

followed this same general procedure in working with young and mature buckwheat. For the mature phase, however, many more cultures than were required for the experiment were grown to the end of the "young" phase in a solution which had proved favorable, and from this excess the necessary number of cultures, uniform apparently insofar as the eye could judge, were selected for the second stage of the work. There are many other investigations which might be cited on this point, but these serve to illustrate the common practice.

The well established fact of biological variation under water-culture conditions has not been ignored, rather various expedients have been employed to minimize it. Thus the rotating table was employed by Shive (7) and others as a means of equalizing environmental influences. Seedlings have been very generally selected for uniform size and vigor and in certain cases [Brenchley (1)] seeds have been graded according to weight. Cultures have usually been made up of several plants, the idea being that less vigorous plants would offset more vigorous ones, and the total weight of any culture would not depart significantly from its replicates. In spite of such precautions, however, variability remains an important factor. Both Brenchley (1) and Stiles (11, 12) recognized that the number of plants which they employed was insufficient to give a mean of more than low accuracy, the former remarking that "owing to the individuality of plants, investigations dealing with small numbers cannot be depended upon," and the latter that, "plants growing in water cultures under exactly the same conditions are very variable." Both of these workers determined the probable error of the mean and interpreted results accordingly, but because of the limited number of determinations averaged and their rather high variability (40 per cent in Brenchley's work), the accuracy of this probable error is very questionable.

American workers have tacitly assumed that under the system of control adopted, variation is reduced to a negligible minimum; in other words, that the mean of few determinations will not depart sufficiently from that of many to vitiate conclusions. One need but examine the literature, however, to find many instances contrary to such an assumption. In many cases noted, where duplicate cultures have been employed, wide differences between them were apparent; in fact, such differences have been frequently observed to exceed that existing between the means under comparison. Where this is true, how much trust can we place in the mean? Certainly, under such conditions, means of duplicates cannot possess any high degree of accuracy, and differences attributed to efficiency of the solution may be due to variability of the plants themselves.

The first consideration, then, in comparing such variables as plants, is the determination of the trustworthiness of the mean; its limit of accuracy must be fixed. This will demand the consideration of a much larger number of replicates than workers have deemed necessary heretofore. Since the distribution of biological variables has been found to follow closely the law of prob-

ability, it is evident that the larger the number of variables averaged, the more accurate will be the arithmetic mean.² On the contrary, small numbers will give means which may depart more or less widely from the true mean, the extent of departure depending on the efficiency of the system.

In the statistical study herein reported, Shive's (7) extended work is taken as a basis. This was selected on account of the very careful attention the author gave to the many details, as well as because it considered the critical comparison of several solutions. Shive employed his well known three-salt solution in three different total concentrations, 0.1, 1.75 and 4.00 atmospheres; (the second of these, the 1.75-atmosphere concentration, is considered here). In these the partial molal concentration of salts was varied in increments of one-tenth, 36 different combinations resulting. A series of 36 cultures, each culture representing different partial concentrations of salts, was set up in 250-cc. bottles, the solutions changed every 3 days, and the experiment continued for 23 days on a revolving table. The greatest care seems to have been taken to have the culture solution the only condition of the environment differing in its influence upon the individual cultures. A careful perusal of the paper did not disclose whether duplicate cultures were set up or not. However, the experiment was repeated at a later date and the means of the dry weights of corresponding cultures in the two series taken for comparison of the efficiency of the solution. Results were plotted by the familiar triangle method as employed in physical chemistry, and on this triangle, areas of high and low yields were mapped out. Lying outside such areas were many results which might be considered medium in their value and these are especially important in a consideration of the data as a whole.

METHODS

In any study of frequency distribution, it is extremely desirable to have a sufficient number of variants to permit of an approximately normal distribution about the mean. In this study, we are dealing only with Shive's 1.75-atmosphere solution, and yet this alone contains 36 different combinations. It would manifestly be difficult to consider all of these statistically, nor is it necessary, since, in order to demonstrate the principle involved, one needs but to consider a few well chosen culture solutions of the 36 represented. For our work, modifications R_5C_2 , R_2C_5 , and R_1C_1 were chosen, these representing his so-called "best," his "poorest," and a "medium" solution,³ the last named lying between the highest and lowest-yield areas. These three had the following partial molal concentrations:

² This, however, as pointed out by Linhart (4), can be true only where the maximum deviation does not exceed the mean in magnitude.

³ The designations are those employed by Shive.

	KH_2PO_4	$\text{Ca}(\text{NO}_3)_2$	MgSO_4
R_1C_2	0.0180	0.0052	0.0150
R_2C_3	0.0072	0.0130	0.0150
R_1C_1	0.0036	0.0026	0.0400

It is likewise true that the number of replicate cultures possible to consider in an experiment of this kind is limited by one's time and equipment, since the changing of solutions each three days for a large number of cultures is a considerable task. For our purposes, two series, "A" and "B", were set up, the first in quart Mason jars and the latter in 250-cc. bottles. Thirty-three cultures (165 plants) for each of the three culture solutions were arranged for Series "A" and 50 (300 plants) for Series "B." Such numbers, of course, were purely arbitrary, but it was felt they would be sufficient for the purpose at hand.

Culture solutions

Baker's analyzed chemicals were employed throughout. Stock solutions of single salts were made up and from these dilutions were made sufficient for a single change. Throughout this work, the importance of subjecting all cultures in any one series to the same culture-solution environment was emphasized and to this end the total amount of solution required for the 33 or 50 cultures, as the case might be, was made up in one lot and this thoroughly mixed several times before being placed in the final containers.

Seedlings

Instead of the Fulcaster wheat used by Shive, Tottingham, McCall, and others, Sonora wheat of selected strain and high germination was employed. This was obtained from the Division of Agronomy of the University of California. Shive's method of germination was used, many more seedlings than necessary being germinated, and only uniform, vigorous seedlings selected for cultures. This latter point was especially stressed, each seedling being carefully measured and all those not coming within the limits of 5 ± 1 cm. rejected.

Glassware

As stated above quart Mason jars were used for series A and 250-cc. wide-mouth bottles for series B. These were cleansed with chromic-sulfuric cleaning mixture, thoroughly rinsed and covered with opaque paper, white side out. Corks were given a very thin coating of "parowax."

Setting up experiments and changing solutions

The two series were set up in the greenhouse, series A on long benches, the position of the jars being changed several times a week in accordance with a

definite plan; series B was placed on revolving tables. Light conditions were excellent and the jars were arranged so as to prevent the shading of one culture by another. Likewise the jars were sufficiently removed from each other to obviate unequal inter-humidity effects. The unheated condition of the greenhouse gave rise to slightly higher variations in day and night temperatures than would have been true had heat been applied (table 1), but such differences were not significantly greater than those reported by Shive and since they affected all plants alike, they were not held to be important.

TABLE 1
Temperatures

	SERIES "A"	SERIES "B"
	°C.	°C.
Mean maxima	26.8	20.8
Maximum	32.5	29.0
Mean minima	9.4	5.2
Minimum	5.5	1.0

In changing solutions, the following procedure was adopted. The total amount of solution required for a single modification in series A, say for R_6C_2 , was 33 liters. This amount was made up from the strong single-salt stock solutions and thoroughly shaken. The cork and plants were placed in an extra bottle containing the solution, the old solution emptied out of the jar and the new lot measured in. The plants were then replaced. In series B the total amount for a change was about 12.5 liters. This was made up in a similar way and the change performed as above. The amount of transpiration was ascertained by measuring the old solution before discarding it.

Criterion of growth

Dry weight of the entire plant and of the top alone was taken as the criterion of growth. After harvesting, the plants were dried at 90°C. for 48 hours, and then to constant weight at 105°C. The weighings for series A were made to 0.01 and in series B to 0.001 gm.

Calculations

The statistical method presented by Davenport (3) and in general use in biometry, was employed except for the calculation of theoretical curves. The latter were calculated according to an equation recently obtained by Dr. G. A. Linhart. Since these methods are taken up *in extenso* by Davenport, by Dr. Linhart (4), and in various texts, it is not thought necessary to go into a mathematical treatment of them here.

TABLE 2

*Total weights, series A**

NUMBER	R ₃ C ₃		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	gm.	gm.	gm.	gm.	gm.	gm.
1	2.64	0.49	2.36	0.22	2.00	0.44
2	2.50	0.35	2.33	0.19	1.57	0.01
3	1.92	0.23	1.96	0.18	1.64	0.08
4	2.06	0.09	2.05	0.09	1.57	0.01
5	2.00	0.05	1.66	0.48	1.76	0.20
6	2.13	0.02	2.47	0.33	1.61	0.05
7	2.09	0.06	1.99	0.15	1.38	0.17
8	2.34	0.19	1.94	0.20	1.53	0.03
9	2.24	0.09	2.33	0.19	1.34	0.22
10	2.06	0.09	2.02	0.12	1.34	0.22
11	2.18	0.03	2.13	0.01	1.57	0.01
12	2.18	0.03	2.13	0.01	1.40	0.16
13	2.27	0.12	2.11	0.03	1.57	0.01
14	2.33	0.18	2.29	0.15	1.80	0.24
15	2.11	0.04	2.13	0.01	1.56	0.00
16	1.83	0.32	1.87	0.27	1.51	0.05
17	2.25	0.10	2.17	0.03	1.75	0.19
18	2.39	0.24	2.04	0.10	1.33	0.23
19	2.12	0.03	2.01	0.13	1.29	0.27
20	1.95	0.20	2.00	0.14	1.49	0.07
21	2.18	0.03	2.19	0.05	1.58	0.02
22	1.71	0.44	2.28	0.14	1.47	0.09
23	1.89	0.26	2.04	0.10	1.45	0.11
24	1.93	0.22	2.15	0.01	1.53	0.03
25	2.27	0.12	2.28	0.14	1.69	0.14
26	2.08	0.07	2.22	0.08	1.57	0.01
27	2.07	0.08	2.23	0.09	1.75	0.19
28	2.23	0.08	2.39	0.25	1.41	0.15
29	1.92	0.23	2.19	0.05	1.62	0.06
30	2.23	0.08	2.46	0.32	1.49	0.07
31	2.46	0.31	1.81	0.35	1.76	0.20
32	2.17	0.02	1.93	0.21	1.75	0.19
33	2.12	0.03	2.46	0.32	1.51	0.05
Mean	2.15 ± 0.023 gm.		2.14 ± 0.023 gm.		1.56 ± 0.018 gm.	
σ	0.194 ± 0.017 gm.		0.195 ± 0.017 gm.		0.156 ± 0.013 gm.	
C.V.	9.02 ± 0.76 per cent		9.10 ± 0.091 per cent		10.0 ± 0.83 per cent	
P.E.	0.131 gm.		0.131 gm.		0.105 gm.	

* 33 cultures, 5 plants per culture; experiment continued 5 weeks; solution changed every 3½ days; results in dry weights per culture.

TABLE 3
Top weights, series A

NUMBER	R ₂ C ₂		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	gm.	gm.	gm.	gm.	gm.	gm.
1	2.04	0.35	1.83	0.12	1.64	0.34
2	1.90	0.21	1.85	0.14	1.31	0.01
3	1.63	0.06	1.54	0.17	1.36	0.05
4	1.52	0.17	1.62	0.09	1.29	0.01
5	1.74	0.05	1.40	0.31	1.45	0.15
6	1.67	0.02	1.98	0.27	1.34	0.04
7	1.68	0.01	1.58	0.13	1.14	0.16
8	1.88	0.19	1.58	0.13	1.29	0.01
9	1.81	0.12	1.80	0.09	1.09	0.21
10	1.67	0.02	1.61	0.10	1.09	0.21
11	1.80	0.11	1.71	0.00	1.25	0.05
12	1.73	0.04	1.74	0.03	1.17	0.13
13	1.73	0.04	1.71	0.00	1.35	0.05
14	1.92	0.23	1.81	0.10	1.59	0.29
15	1.75	0.06	1.72	0.01	1.35	0.04
16	1.35	0.34	1.51	0.20	1.27	0.04
17	1.76	0.07	1.76	0.05	1.46	0.16
18	1.84	0.16	1.62	0.09	1.12	0.18
19	1.63	0.06	1.65	0.06	1.08	0.22
20	1.53	0.16	1.54	0.17	1.25	0.05
21	1.67	0.02	1.70	0.01	1.31	0.01
22	1.37	0.32	1.78	0.07	1.25	0.05
23	1.50	0.19	1.61	0.10	1.19	0.11
24	1.58	0.11	1.71	0.00	1.27	0.03
25	1.71	0.02	1.86	0.15	1.40	0.10
26	1.62	0.07	1.79	0.08	1.27	0.03
27	1.66	0.03	1.81	0.10	1.44	0.14
28	1.78	0.09	1.90	0.19	1.16	0.14
29	1.48	0.21	1.72	0.01	1.30	0.00
30	1.87	0.18	1.87	0.16	1.27	0.03
31	1.48	0.21	1.48	0.23	1.48	0.18
32	1.55	0.14	1.55	0.16	1.46	0.16
33	1.98	0.31	1.98	0.27	1.33	0.03
Mean	1.69 ± 0.018 gm.		1.71 ± 0.017 gm.		1.30 ± 0.016 gm.	
σ	0.153 ± 0.013 gm.		0.14 ± 0.012 gm.		0.135 ± 0.011 gm.	
C.V.	9.05 ± 0.76 per cent		8.24 ± 0.69 per cent		9.66 ± 0.80 per cent	
P.E.	0.10		0.094		0.090	

DISCUSSION OF DATA

It is stated in the law of errors that when a number of variants are considered, large deviations from the mean will occur relatively less frequently than small ones; moreover, when a sufficiently large number of such variants

are taken, deviations will fall equally on either side of the mean. This is true, of course, only under ideal conditions where chance is not interfered with in any way. One can plot such data, frequency against measurement, and obtain the so-called frequency curve, the apex of a normal curve representing the true mean and departures from that mean falling equally on either side. In the case of a small number of variants, it is not likely that perfect symmetry would be obtained, since the law of chance would not have fair play, and more experimental values might fall on one side of the true mean than on the other. This would give the so-called "skew," or asymmetric curve. Such unbalanced distribution is not peculiar to few variants. Indeed, it is a common arrangement of biological data even where many variants are considered. In the data given in this paper, the deviation of individual determinations from the mean is relatively small, i.e., it never exceeds the mean in magnitude, while the distribution of such determinations about the arithmetic mean is in all cases such as to permit the construction of a symmetrical curve (fig. 5 to 10).

In case the extent of one's experimental data falls short of the ideal for the symmetrical curve (the usual condition), and yet is sufficiently extensive to give an approximation of symmetry, the normal curve can be calculated, and thus the experimental can be related to theoretical values. It is obvious that in such a comparison, the arithmetic mean may not coincide with the theoretical as indicated by the apex of the calculated curve; if there is a divergence, it must be assumed that the theoretical mean is the true one, since the law of probability demands symmetry. It becomes entirely possible, then, that one may fall into error in comparing different series of determinations through their arithmetic means alone, even although a relatively large series may be considered, since these may neither indicate the true mean nor show the influence of variability. The chances for misleading conclusions become much greater, however, when few determinations are averaged, particularly when stress is laid upon slight differences, for here, as will be shown later, the arithmetic mean may vary greatly from the more nearly true mean of a large series. In the data herein presented, the variants considered, when grouped into classes give approximation of symmetry to the plotted frequency polygon, and from these, normal curves have been calculated which show graphically the extent of variability. Probable error,⁴ standard deviation, and the coefficient of variability, have been determined for plants grown in

⁴ The probable error of a single determination is given as that value lying on either side of the mean within which fifty per cent of the determinations of the series should lie. To find the probable error of a number of variants, use is made of the standard deviation, which is expressed as $\sigma = \frac{\sum d^2}{N}$, where $\sum d^2$ is the sum of the squared deviations, N the number of variants, and σ the standard deviation. The probable error, PE , is then found by the formula $P.E. = 0.6745 \frac{\sigma}{\sqrt{n}}$, where 0.6745 is a constant. [See Davenport (3) and Merriam (6).]

each of the three solutions, and comparisons made in the light of these limiting factors. Assuming that the mean of any one solution series is accurate within its probable error,⁵ we have a basis for judging the reliance which can be placed upon the means of duplicate cultures, or of any number which may be chosen at random from the series in question.

Series A

In this series, the culture (5 plants) rather than the individual plant is taken as a unit, thus following the common practice. Later, the variability shown by individual plants will be treated separately. The experimental data are given in tables 2 and 3, and are self-explanatory.

In Shive's original results, the values obtained for the means of two cultures are given as follows:

	R_2C_2	R_2C_1	R_1C_1
Dry weight, gm.....	0.5704	0.4842	0.4104
With R_1C_1 as unity.....	1.39	1.18	1.00

Referring to table 2, however, we find the means for 33 cultures of R_2C_2 and R_2C_1 practically coincide. This is graphically shown in figures 1 and 2 where the corresponding calculated curves are compared. The curves show the existence of considerable variability for the cultures in question, which has resulted in an overlapping of data; in the case of the first two solutions being practically complete, and with R_1C_1 relatively slight. Even under the accepted conditions of control, the range due to variation is seen to be considerable and offsets completely any apparent difference between arithmetic means. It is evident that were we comparing means of duplicates, our conclusions might have been otherwise, since they would be influenced by the position occupied by the cultures represented within the area of the curve in question. A glance at the figures will show at once that such chance distribution might give results comparable to those of Shive, to those herein presented, or different from either in that R_2C_1 might be concluded the better solution. This point will be considered more in detail later.

In the case of R_1C_1 , we have a solution high in magnesium sulfate and relatively low in nitrate and phosphate salts. *A priori*, one would expect a yield lower than that given by the other two solutions, conditions which are realized in the analysis of the results. The mean of 33 cultures is here 1.56 gm. as

⁵ The mean of a number of variants cannot have a fixed value; the very fact of measurement introduces error. In biological data there is not only the error of measurement which may be relatively small, but errors as well due to an innate capacity to vary on the part of the organism. One must not only consider the mean in his calculations, but the probable error as well, since this, in the last analysis, fixes its accuracy. See Wood (14), Wood and Stratton (15), Merriam (6), Davenport (3) and texts on the law of probability, for discussion.

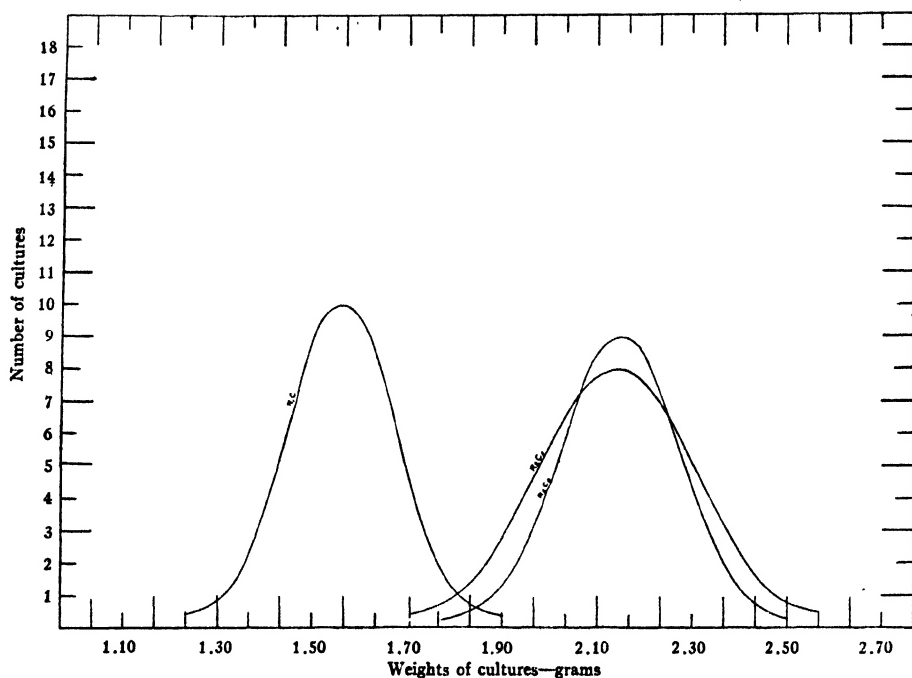


FIG. 1. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL CULTURES. SERIES A

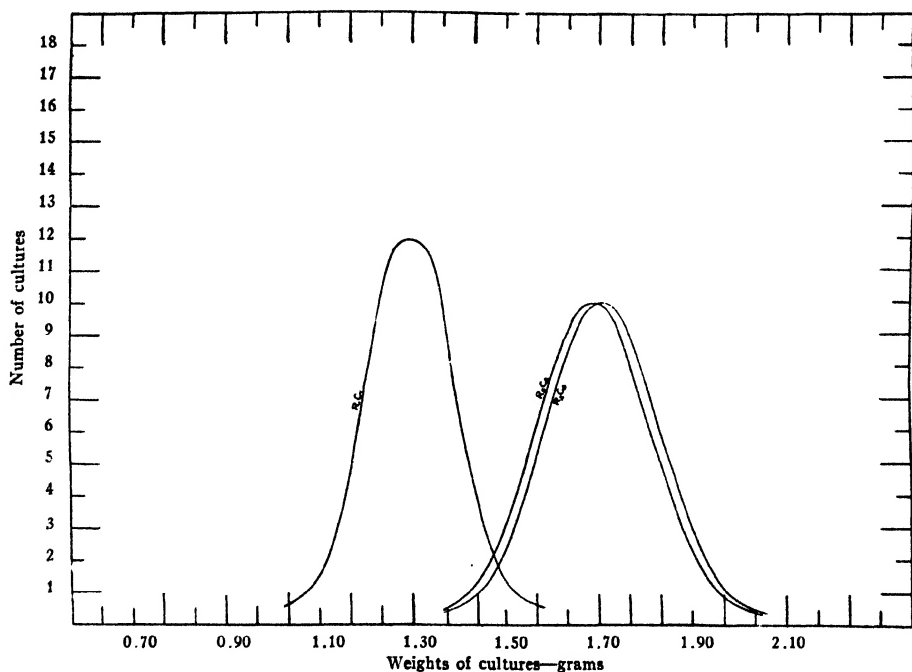


FIG. 2. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF WEIGHTS OF TOPS, INDIVIDUAL CULTURES. SERIES A

compared with 2.15 gm. for R_5C_2 , a difference of 0.59 gm., or about 27 per cent in terms of the latter solution. This difference is quite marked, and one might be justified in attaching significance to it. Referring to the curves in figures 1 and 2, however, it is seen that the overlapping, although slight, is such as to raise the question of its relative importance. How can one be certain that small numbers of cultures chosen at random from the series might not lie within this overlapping area? Since the ratio of this area to the area of the curves compared is small, it would be supposed that the chances for such an event occurring would be correspondingly slight. It is possible, however, to determine such odds when the probable error of difference between means is known.⁶ Thus, in this case, for example, we have the means of these two solutions as 2.15 ± 0.023 gm. and 1.56 ± 0.018 gm., respectively, and by the formula given, we find this difference of 0.59 gm. has a probable error of 0.029 gm.

TABLE 4
Table of odds—Differences in both directions

DIFFERENCE FROM THE MEAN IN TERMS OF PROBABLE ERROR	DIFFERENCE BETWEEN TWO RESULTS IN TERMS OF THE PROBABLE ERROR OF EACH RESULT	ODDS AGAINST SUCH DIFFERENCE OCCUR- RING UNDER UNIFORM CONDITIONS
1.00	1.41	1 to 1
1.25	1.76	3 to 2
1.44	2.03	2 to 1
1.71	2.41	3 to 1
1.90	2.68	4 to 1
2.00	2.83	9 to 2
2.05	2.87	5 to 1
2.50	3.53	10 to 1
2.93	4.13	20 to 1
3.00	4.24	22 to 1
3.20	4.51	30 to 1
4.00	5.66	140 to 1
4.90	6.93	1000 to 1
5.00	7.07	1350 to 1

Thus the actual difference between means is about 19 times its probable error. The significance of this value can be ascertained by reference to a table of odds, such as those included here under tables 4 and 5 which have been taken from Wood (14). In this case, the difference lies in one direction only, i.e., the effect of such a solution as R_1C_1 can be considered as varying in but one direction from R_5C_2 , and so table 5 must be utilized. Taking the value 19 to the second column of this table, the corresponding odds in the third column, in favor of this difference being due to something other than normal variation, would be seen to be enormous were they computed to so high a figure. Cer-

⁶ The probable error of difference is larger than that of either probable error alone, and is given by the formula: $A_2 - A_1 \pm \sqrt{E_1^2 + E_2^2}$, where A_1 and A_2 are the means, and E_1 and E_2 their respective probable errors.

tainly here this overlapping need not be considered and real inferiority may be assigned to the solution.

The above conclusions are made on the basis of 33 cultures where chance has had somewhat of an opportunity to locate a true mean. What would be the result were one to consider duplicates? In decreasing the number of cultures, the most apparent fact is the increase in the probable error, which

TABLE 5
Table of odds—Difference in one direction only

DIFFERENCE FROM THE MEAN IN ONE DIRECTION ONLY IN TERMS OF PROBABLE ERROR	DIFFERENCE BETWEEN TWO RESULTS IN ONE DIRECTION ONLY IN TERMS OF THE PROBABLE ERROR OF EACH RESULT	ODDS AGAINST SUCH DIFFERENCES OCCURRING UNDER NORMAL CONDITIONS
1.00	1.41	3 to 1
1.25	1.76	4 to 1
1.44	2.03	5 to 1
1.58	2.23	6 to 1
1.71	2.41	7 to 1
1.81	2.55	8 to 1
1.90	2.68	9 to 1
2.00	2.83	10 to 1
2.48	3.50	20 to 1
2.70	3.81	30 to 1
2.89	4.07	40 to 1
3.00	4.24	44 to 1
3.03	4.28	50 to 1
3.44	4.85	100 to 1
4.00	5.66	290 to 1
5.00	7.07	2700 to 1

TABLE 6

Increase in probable error of the mean, with the decrease in the number of cultures—Series A

NUMBER OF CULTURES	TOTAL WEIGHT			TOP WEIGHT		
	R ₅ C ₁	R ₂ C ₅	R ₁ C ₁	R ₅ C ₂	R ₂ C ₅	R ₁ C ₁
	gm.	gm.	gm.	gm.	gm.	gm.
2	2.15-0.095	2.14-0.095	1.56-0.075	1.69-0.071	1.71-0.066	1.30-0.067
4	2.15-0.066	2.14-0.065	1.56-0.052	1.69-0.050	1.71-0.047	1.30-0.043
8	2.15-0.047	2.14-0.047	1.56-0.038	1.69-0.036	1.71-0.033	1.30-0.033
16	2.15-0.034	2.14-0.034	1.56-0.026	1.69-0.025	1.71-0.023	1.30-0.023
33	2.15-0.025	2.14-0.023	1.56-0.018	1.69-0.018	1.71-0.016	1.30-0.016

varies as the square root of the number of cultures averaged. This is shown in table 6, where probable errors have been calculated for various numbers of cultures up to the 33 making up the series. Here it is seen that where the probable error of the mean total weights of both R₅C₂ and R₂C₅ is 0.023 gm., it increases to 0.095 gm. for duplicate cultures within these two series. Should we select any two cultures at random from either R₅C₂ or R₂C₅, their average by our definition of probable error would have but an even chance of coming

within the limits 2.05–2.25 gm. When we undertake to compare means of such low degree of accuracy as is exhibited here, it is perfectly evident that the difference between them must be considerable before we would be justified in assigning even relative efficiency values to these solutions. In the case at hand, it is possible to determine just how great this difference must be. We have found that the probable error of difference between R_5C_2 and R_5C_5 becomes 0.134 gm. when the comparison is made through the means of duplicate cultures. To obtain an even chance that a difference is real, it must equal the probable error of difference in magnitude, or in this case 0.134 gm. For the security of a 10 to 1 chance (table 4), a difference between solutions must exist equal to 2.83 times the probable error of difference (column 2), or in this case, $2.83 \times 0.134 = 0.379$ gm. However, a 10 to 1 chance is not considered sufficient to give security of the degree desired in this sort of work. It is more customary to demand the reliance of 30 to 1 odds, and if this were desired, one would not be justified here in attaching significance to a difference less than 0.53 gm., or 24 per cent of the mean.

It was noted above that the inferiority of R_1C_1 , as shown by the mean of the series, was most evident. Is the larger probable error when duplicates are compared, sufficient to offset this difference in arithmetic means? The probable error increases to 0.078 gm. for duplicates and when this solution is compared with duplicates of R_5C_2 , we find a difference of 0.59 ± 0.13 gm. The ratio of difference to probable error becomes 4.54, which, when taken into the second column (table 5) gives chances approximately 30 to 1 that the difference is due to something other than normal variation.

COMPARISON OF TOP WEIGHTS

There is considerable divergence of opinion among investigators regarding the relative value of total dry, top dry, or green weight, as a criterion of solution efficiency. The two former are employed as criteria here and when the means of 33 cultures are compared, no real difference in relative results is apparent (tables 2 and 3). In the comparison of top weights for duplicate cultures, however, the difference between R_1C_1 , and R_5C_2 becomes less pronounced than where total weights were compared, thus:

$$1.69 - 1.30 \pm \sqrt{80^2 \text{ mgm.} + 67^2 \text{ mgm.}} = 0.39 \pm 0.105 \text{ gm.,}$$

$$\text{and } \frac{0.39}{0.105} = 3.71$$

This value gives less than a 20 to 1 chance that the difference is significant. This, again, illustrates the error of considering too few cultures. The striking disparity between the means of these two solutions indicates a real difference, but because of overlapping due to variation, and the low accuracy of means of duplicates, one cannot have a chance better than 20 to 1 that it is real, unless the probable error is decreased by averaging a greater number of cultures. Thus, for 33 cultures, the probable error of difference decreases to 0.026 gm., and shows without question the real inferiority of the solution.

TABLE 7
Total weight, series B*

NUMBER	R ₃ C ₂		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	534	59	440	20	422	2
2	484	9	487	27	432	12
3	582	107	525	65	430	10
4	468	7	471	11	428	8
5	489	14	481	21	470	50
6	507	32	482	22	452	32
7	483	8	479	19	454	34
8	424	48	408	52	458	38
9	525	47	417	43	380	40
10	545	65	464	4	375	45
11	547	18	461	1	425	5
12	428	47	449	11	428	8
13	465	10	472	12	390	30
14	504	29	469	9	408	12
15	482	7	544	84	461	41
16	430	45	460	0	436	16
17	476	1	482	22	398	12
18	418	59	394	66	413	7
19	445	35	383	77	452	32
20	385	90	443	17	426	6
21	455	20	452	78	412	8
22	492	17	457	3	430	10
23	494	19	451	9	361	59
24	513	38	517	57	421	1
25	412	63	522	62	365	55
26	424	49	411	49	434	14
27	431	44	459	1	428	8
28	504	30	455	5	408	12
29	404	68	479	19	340	80
30	453	22	426	34	459	39
31	427	48	538	78	376	44
32	536	61	451	9	400	20
33	484	11	424	36	440	70
34	524	45	454	6	438	18
35	509	34	438	96	458	38
36	404	67	479	19	362	58
37	434	41	389	71	422	2
38	527	52	390	70	424	4
39	452	23	448	12	440	20
40	520	45	458	2	425	5
41	561	86	494	34	383	37
42	465	16	411	49	436	16
43	468	7	509	49	461	41

* 50 cultures, 6 plants per culture; experiment continued 23 days; solution changed every 3 days; results in dry weights per culture.

TABLE 7—*Continued*

NUMBER	R_5C_2		R_2C_5		R_1C_1	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
44	510	51	504	44	313	107
45	505	29	477	17	483	63
46	445	38	460	0	500	80
47	425	51	433	27	416	4
48	445	37	455	5	369	51
49	489	17	498	38	423	3
50	510	35	440	20	445	25
Mean	475 \pm 4.3 mgm.		460 \pm 3.8 mgm.		420 \pm 3.5 mgm.	
σ	44 \pm 3.0 mgm.		40 \pm 2.7 mgm.		37 \pm 2.5 mgm.	
C.V.	9.30 \pm 0.62 per cent		8.70 \pm 0.58 per cent		8.81 \pm 0.59 per cent	
P.E.	30 mgm.		27 mgm.		25 mgm.	

SERIES B

Total weight

In this series, attempt was made to duplicate as nearly as possible the methods and technique employed by Shive. The quart jars used in the preceding series were replaced by 250-cc. wide-mouth bottles; solutions were changed every 3, instead of every $3\frac{1}{2}$ days, and the experiment was carried on for 23 days, instead of for 5 weeks. Six plants made up a culture and 50 cultures were set up rather than 33. The average temperature was lower than for series A (table 1), and this, together with the shortened growing period, resulted in lower yields. Other possible temperature influences will be discussed later.

Reference to figures 3 and 4 clearly shows the variation existing within each of the three solutions and the overlapping due to this variation. While the means for R_5C_2 and R_2C_5 do not approach each other so closely as is true in series A, the three curves considered together are more closely associated than in that series. The value of such curves is here demonstrated. Were one to consider separate means without taking variation into account, the conclusion might be reached that the three solutions showed real differences. However, where the data are plotted in the form of a curve and the variation shown graphically, one can see at a glance that the question of overlapping, as caused by variation, must be seriously considered.

Table 7 shows the means of the three solutions in this series to be 475 \pm 4.3 mgm., 460 \pm 3.8 mgm., and 420 \pm 3.5 mgm., for R_5C_2 , R_2C_5 , and R_1C_1 , respectively. In this case, an appreciable difference seems to exist between the means of R_5C_2 and R_2C_5 , the relative difference, in fact, being approximately that noted by Shive. Clearly, if arithmetic means alone are compared, the latter solution must be assigned an efficiency value less than that of R_5C_2 .

TABLE 8
*Top weights, series B**

NUMBER	R ₂ C ₂		R ₂ C ₃		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	359	36	286	14	291	1
2	343	20	309	9	309	19
3	409	86	357	43	297	7
4	328	5	320	20	299	10
5	380	57	333	33	320	29
6	344	21	323	23	311	21
7	332	10	322	22	313	23
8	283	60	281	19	307	17
9	352	29	286	14	251	39
10	350	27	330	30	256	34
11	313	10	307	7	288	2
12	290	33	294	6	282	8
13	316	7	311	11	262	28
14	332	11	305	5	282	8
15	319	4	349	49	311	21
16	300	23	313	13	305	15
17	332	11	315	15	277	13
18	272	51	265	35	283	7
19	300	23	257	43	321	31
20	272	51	294	6	292	2
21	317	6	306	48	277	13
22	349	26	321	21	305	15
23	347	24	300	0	253	37
24	343	20	338	38	295	5
25	286	37	316	16	263	27
26	293	32	282	18	304	14
27	305	18	298	2	302	12
28	332	11	300	100	279	11
29	263	60	324	18	235	55
30	282	41	289	17	320	29
31	306	17	359	53	269	21
32	350	27	309	3	284	6
33	330	7	282	24	218	28
34	355	32	310	4	257	33
35	342	19	294	12	318	28
36	278	45	308	2	247	43
37	302	21	255	51	316	26
38	354	31	269	37	297	7
39	312	11	305	1	309	19
40	345	22	318	12	299	9
41	384	61	329	23	247	43
42	314	9	263	53	297	7
43	322	1	339	33	321	31

* 50 cultures, 6 plants per culture; experiment continued 23 days; solutions changed every 3 days; results in dry weights per culture.

TABLE 8—Continued

NUMBER	R ₄ C ₂		R ₂ C ₄		R ₁ C ₁	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
44	297	26	328	22	206	84
45	339	16	307	1	329	39
46	310	13	303	3	345	55
47	288	35	284	22	282	8
48	298	25	296	10	266	24
49	340	17	339	33	286	4
50	341	18	284	22	299	10
Mean	323 ± 3.0 mgm.		306 ± 2.3 mgm.		290 ± 2.9 mgm.	
σ	31 ± 2.1 mgm.		24 ± 1.6 mgm.		30 ± 2.01 mgm.	
C.V.	9.60 ± 0.64 per cent		7.84 ± 0.52 per cent		10.35 ± 0.69 per cent	
P.E.	20.8 mgm.		16.1 mgm.		20.1 mgm.	

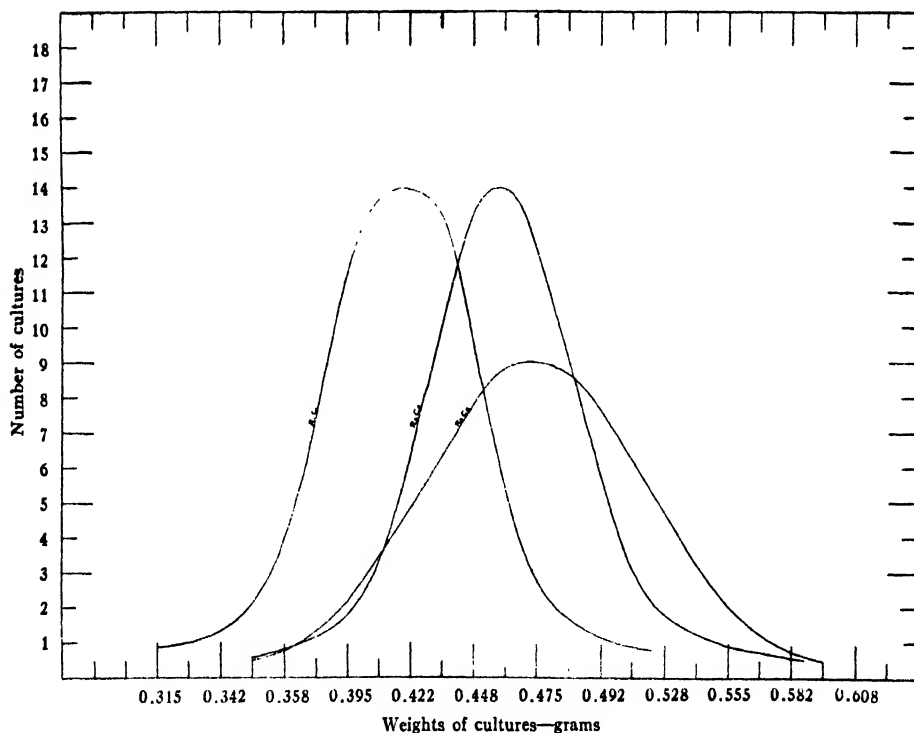


FIG. 3. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL CULTURES. SERIES B

In comparing R_5C_2 and R_2C_5 , we find a difference of

$$475 - 460 \pm \sqrt{4.3^2 + 3.8^2},$$

$$\text{or, } 15 \pm 5.8 \text{ mgm.}$$

We do not know whether such a difference will always occur in the same direction, and hence, in the computing of odds, recourse must be had to table 4. The difference obtained is 2.58 times the probable error, a value which represents but a 4 to 1 chance that the discrepancy in means is due to a fundamental difference in solution and not to variability. To insure odds of

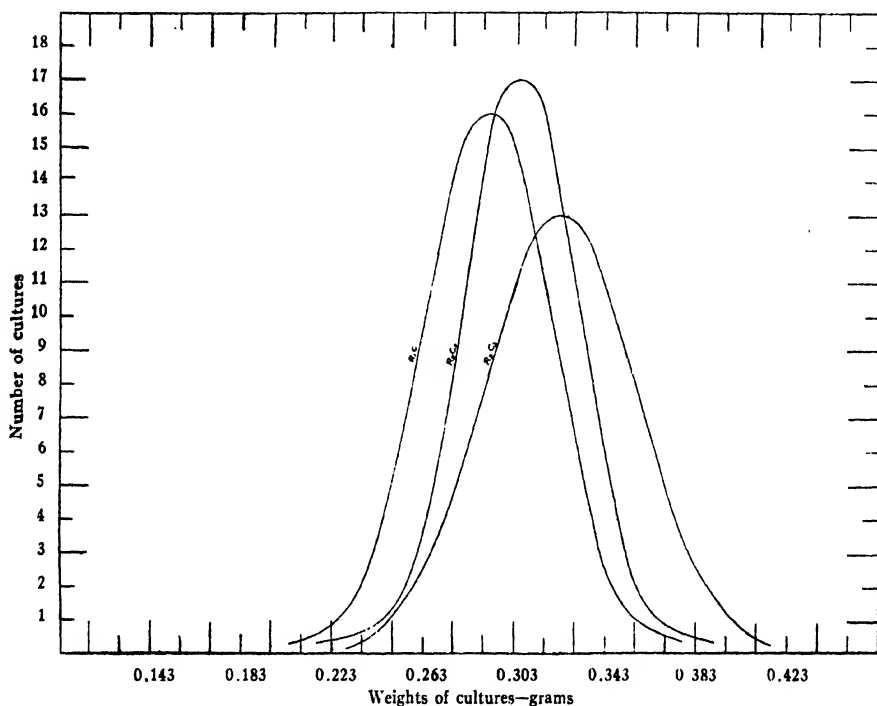


FIG. 4. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL CULTURES. SERIES B

30 to 1, the probable error of difference must be reduced to 3.3 mgm., or with the probable error as found a difference of at least 26 mgm. between the two means must exist. The disparity in means of R_5C_2 and R_1C_1 , is again sufficiently great to warrant the previous conclusions regarding its inferiority, its value being 55 ± 5.7 mgm.

From table 9, we find the probable errors of duplicate cultures become 21 mgm., 19 mgm., and 18 mgm., for the three solutions in order. Once more, there is but a 1 to 1 chance that the mean of any two cultures will fall within the probable error of the mean of the series. In the case of R_5C_2 and R_2C_5 , the difference in means is 15 ± 28 mgm. It is difficult indeed, to say that

this value means anything definite, for the probable error is of such magnitude as to annul completely the significance of difference. The question might arise that since there is an apparent difference between the solutions, and the probable error decreases with the number of cultures employed, all that is necessary is to decrease the probable error until such a point is reached as will give the security of chance desired. This might be done if one wishes to lay emphasis upon such small differences. It must be remembered, however, that the probable error decreases rapidly at first, then more and more slowly as the number of cultures is increased, and, to attain the accuracy desired, the number of cultures required might become prohibitive.

TABLE 9

Increase in the probable error of the mean, with the decrease in the number of cultures—Series B

NUMBER OF CULTURES	TOTAL WEIGHT			TOP WEIGHT		
	R_3C_2	R_2C_3	R_1C_1	R_3C_2	R_2C_3	R_1C_1
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
2	475±21.0	460±19.0	420±18.0	323±15.0	306±11.4	290±14.2
4	475±15.0	460±14.0	420±13.0	323±11.0	306±8.0	290±10.0
8	475±11.0	460±9.8	420±9.0	323±7.4	306±5.8	290±7.2
16	475±7.3	460±7.0	420±6.2	323±5.2	306±4.0	290±5.0
32	475±5.3	460±4.8	420±4.4	323±3.6	306±2.8	290±3.5
50	475±4.3	460±3.9	420±3.6	323±3.0	306±2.2	290±2.8

THE RELATION OF THE MEAN OF DUPLICATE CULTURES CHOSEN INDISCRIMINATELY, TO THE MEAN OF THE SERIES IN WHICH THEY LIE

It has been shown above that odds in favor of the difference between two means being significant, decrease with a decrease in the number of samples averaged. Thus, for duplicate cultures, greater differences between the means of two pairs must be apparent than would be true for larger numbers. As stated in the introduction, the worker who employs duplicate cultures for comparative purposes assumes that no matter how many sets of duplicates he might use, the means would not vary enough from each other to vitiate his conclusions. The error of this assumption is most evident from the data in table 10. Here we have the mean of 33 cultures from series A as a check, and can select any number of pairs desired to see whether they approach this more accurate mean within reasonable limits. Sixteen pairs of cultures were selected by chance from the data for R_3C_2 of this series. Here, in a single solution and under a uniform set of conditions for all cultures, the means of these pairs so chosen range from 2.55 gm. to 1.82 gm., or 34 per cent of the mean of the series, 2.15 gm. Had we based our comparisons upon one of these random means and upon another chosen similarly from solution R_2C_3 , it is seen that the conclusions reached would have depended entirely upon the mean which chance happened to throw in our way. It might be stated

however, that, when sampling at "random," the chances of getting a pair of values representing a large deviation from the true mean are not so great as they would be for a small deviation. In this case, we may take the probable error of two cultures, which has already been calculated for solution R_5C_2 , i.e., 0.095 gm. Any haphazard selection, then, of the means of a pair of weights, such as given in the table, would have but a 1 to 1 chance of coming within the limits of 2.15 ± 0.095 gm. When we group these selected means in table 10 according to the relative size of their errors, we actually find that seven are found within such limits and nine outside, which is a rough approximation of the theoretical odds. The fact is here apparent in this experiment that one time out of two, we shall fall short of the true mean by at least one-tenth of a gram. Thus one would hardly be justified in attaching significance to a difference in the second, third, or fourth decimal place.

TABLE 10

Variations shown in the means of duplicate cultures chosen at random from series A

SELECTION NUMBER	VARIATION	SELECTION NUMBER	VARIATION
	gm.		gm.
1	2.22	9	1.97
2	2.18	10	2.05
3	1.97	11	2.37
4	2.15	12	2.25
5	2.34	13	2.09
6	2.00	14	2.55
7	2.16	15	1.99
8	2.28	16	1.82
Mean.....			2.15

VARIABILITY AS SHOWN BY INDIVIDUAL PLANTS

Although, as previously noted, certain workers have employed cultures of one plant each as the experimental unit, the common practice has been to use several plants to the culture. Conforming to this custom, we have in the foregoing discussion considered the culture as the basis for statistical treatment. It is perfectly obvious, however, that such collective units do not express the true variability of the individual plants, since the very act of grouping tends to offset any extreme errors which might be exhibited by the individuals alone. The literature has very little to say on this point as applied to water cultures. Brenchley (2) and Stiles (11), determined the probable error of the mean of 10 one-plant cultures. So few plants, however, cannot disclose the full extent of individual variation.

A difference of opinion exists regarding the relative advantages of cultures made up of one or several plants. The first condition gives optimum conditions for growth as far as interference by other plants is concerned, but the advantages of an average of several plants is lost.

The individual plants of series A were weighed separately and will here be treated as statistical units. Such data have not been placed in tabular form; it has been considered advantageous to treat them directly in connection with

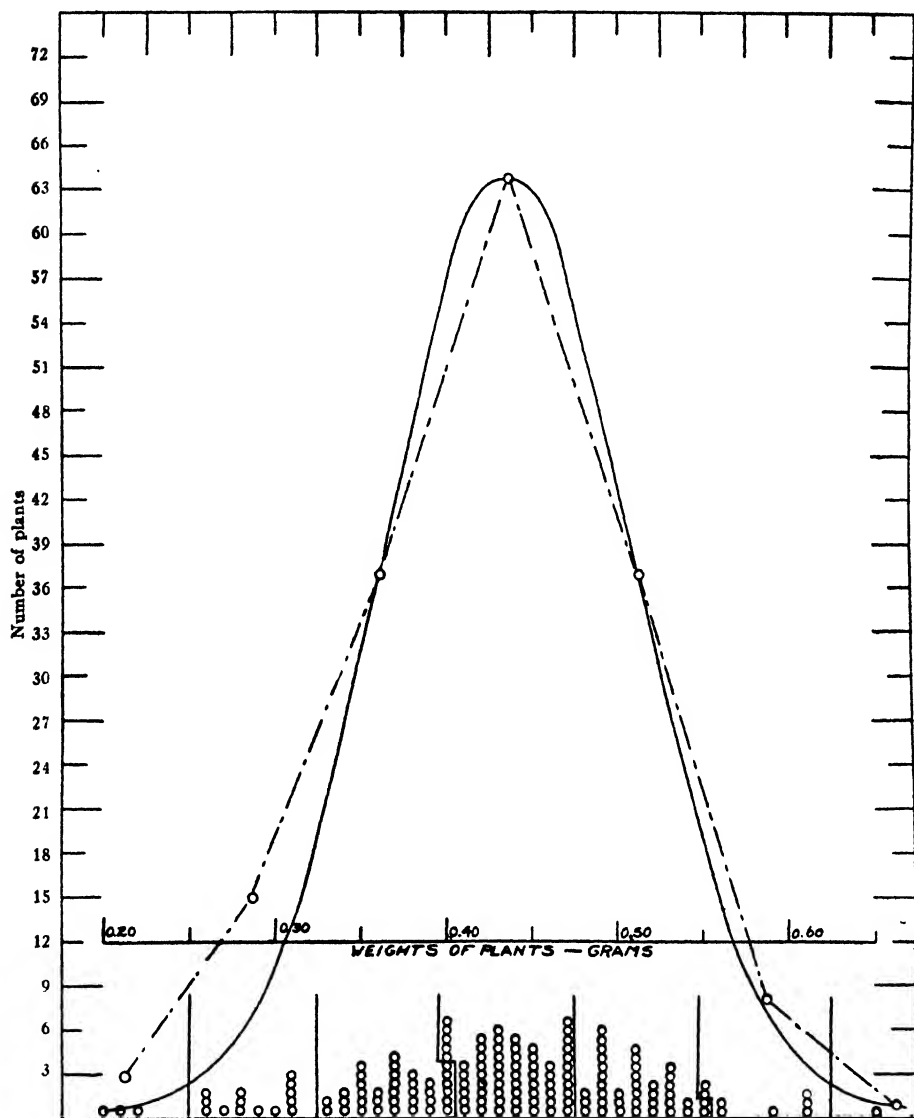


FIG. 5. SOLUTION R_6C_2 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

the constructed curves. Thus figures 5 to 10 show not only the polygons for experimental data (broken lines), but the calculated frequency curves and the original determinations from which the curves were constructed. These latter are shown as small circles at the base of the polygon making it possible to read

off directly the value of every determination of the 165 considered. In figure 5, for example, the separate determinations for R_5C_2 are so indicated. In plotting the experimental polygon, the weight values have been grouped

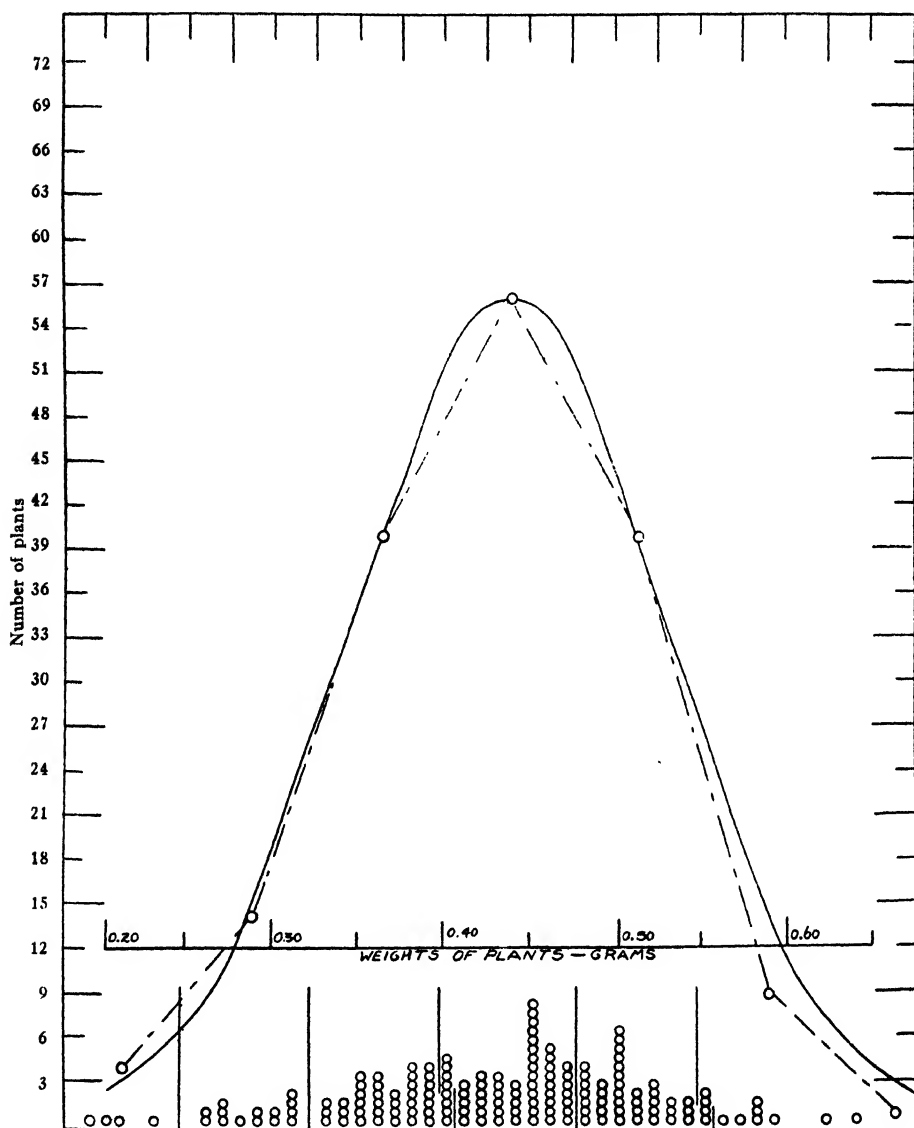


FIG. 6. SOLUTION R_2C_6 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

into classes, the distribution being such as to give an approximation of a normal curve. These classes are indicated by the heavy separating lines at the base of the figure, and the frequencies of determinations included in them are shown by the ordinate values. The various calculated values for the six

curves are given in table 11 where they may be compared. It is seen that here, as in the original series A the mean weights for solutions R_5C_2 and R_2C_5 are the same. In this case, the curve for R_2C_5 is the flatter of the two, show-

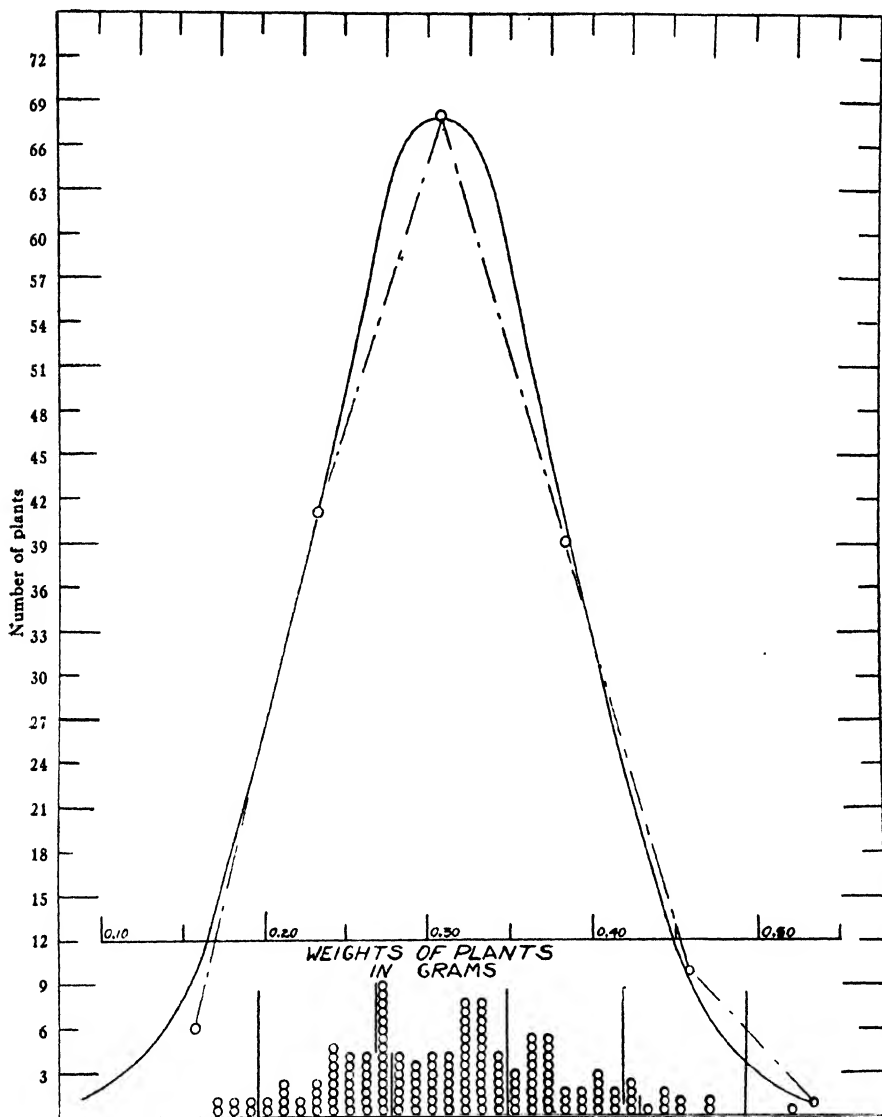


FIG. 7. SOLUTION R_1C_1 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

ing a wider range, i.e., a larger coefficient of variability. In fact, these coefficients of variability for both total weights and tops are shown to increase inversely as the values attributed to the solutions by Shive (7). Whether this fact is significant or not remains to be shown. It might indicate that

the real difference between these two solutions lies in the extent of variability which they exhibit rather than in their means. If this be true, R_3C_3 might be called less efficient than R_3C_2 . However, we know practically nothing regard-

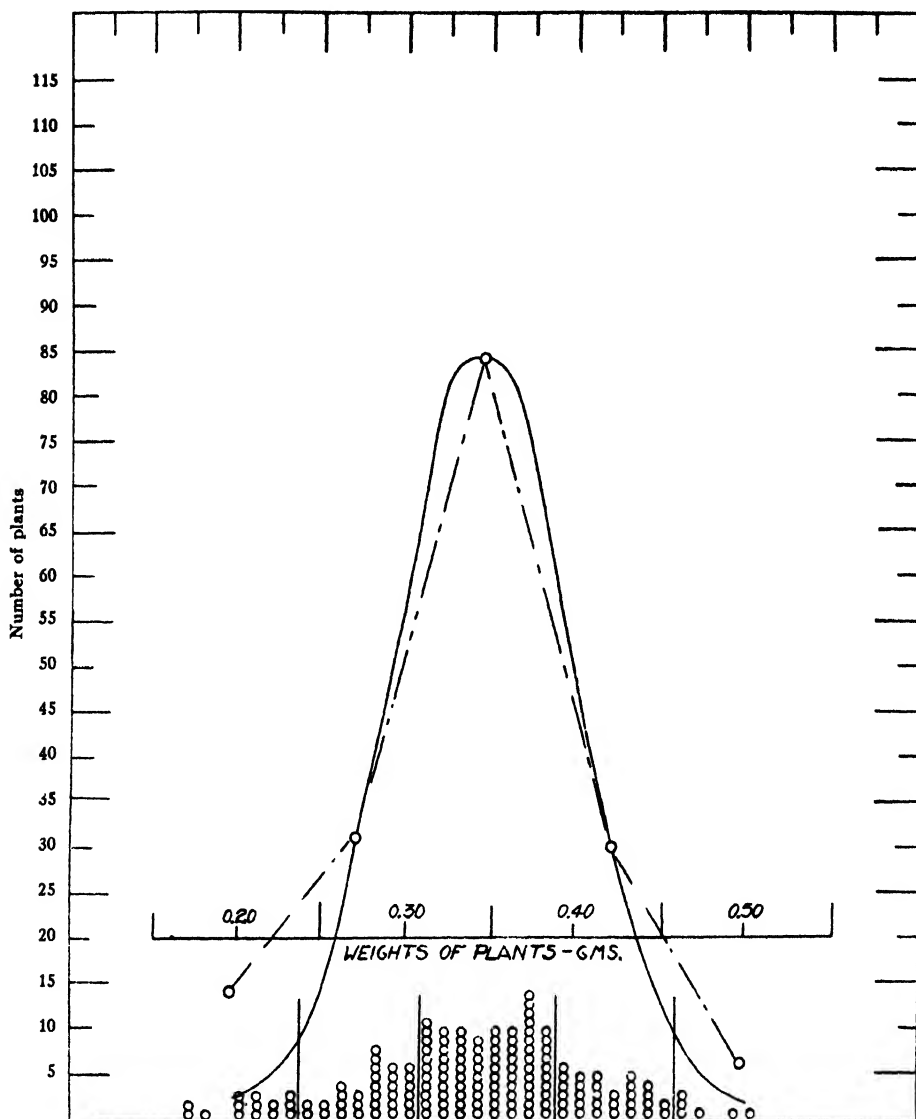


FIG. 8. SOLUTION R_6C_2 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

ing the effect of different culture solutions upon the variability of plants grown in them and certainly at the present time are not warranted in drawing distinctions on such a basis. Further study is required on this point.

In figures 11 and 12, this more extensive variation shows itself in an increased overlapping of the three curves as compared to that evident in figures 1 and 2. The difference between R_5C_2 and R_1C_1 remains significant, however, even

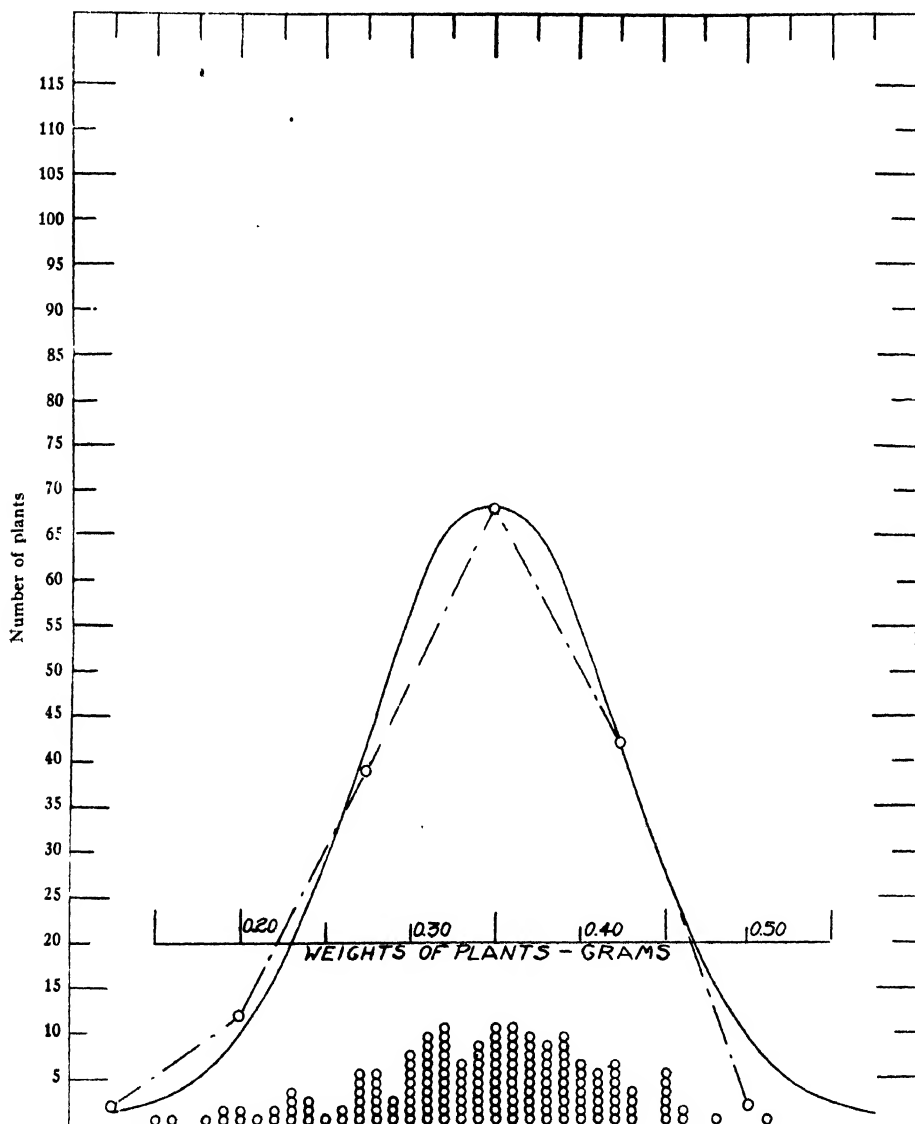


FIG. 9. SOLUTION R_2C_5 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

when compared through the probable error of 10 plants. This probable error becomes 11 mgm. for R_5C_2 , and 12 mgm. for R_1C_1 and from these the probable error of difference on the basis of 10 plants is ± 16 mgm.

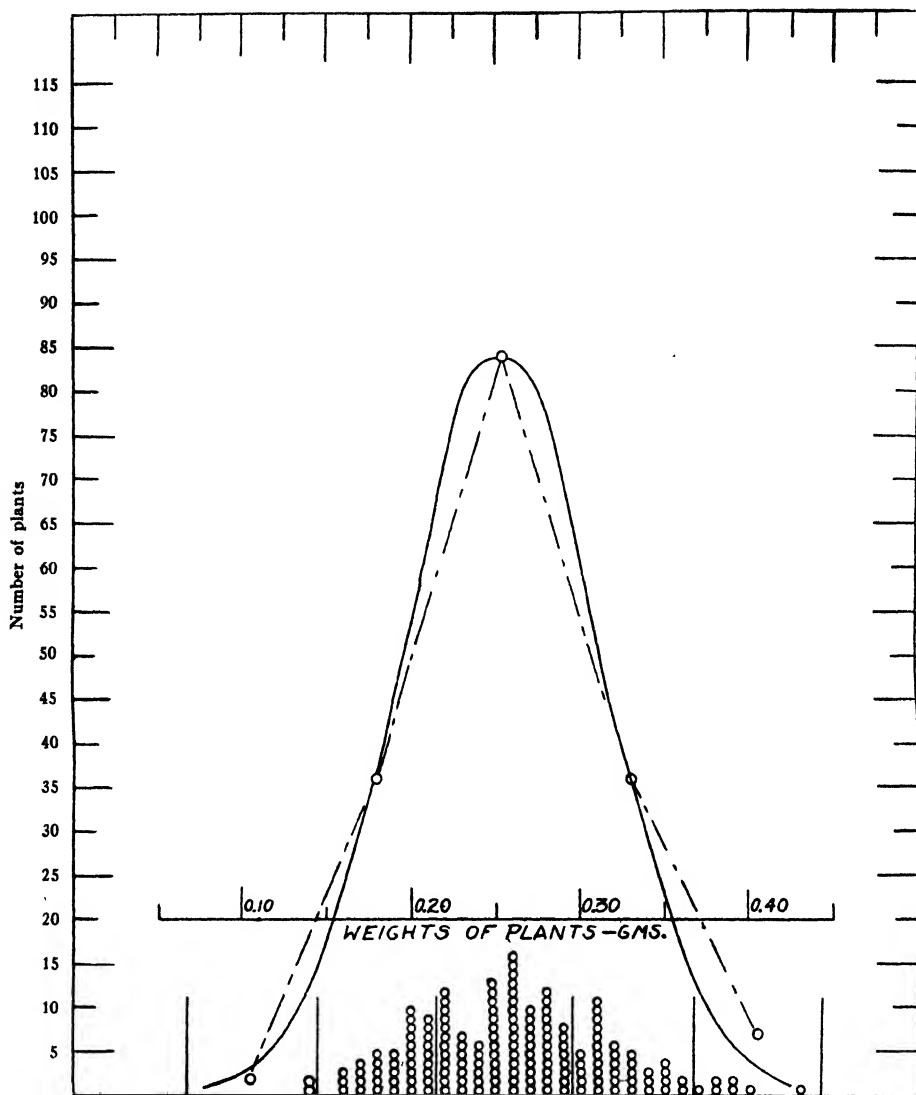


FIG. 10. SOLUTION R_1C_1 ; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

TABLE 11

Condensed table of values, individual plants—Series A

	WEIGHT OF COMPLETE PLANT			WEIGHT OF TOPS		
	R_1C_2	R_2C_1	R_1C_1	R_2C_2	R_3C_1	R_1C_1
Mean (mgm.).....	438 ± 3.0	438 ± 4.0	310 ± 3.9	350 ± 4.0	350 ± 4.8	260 ± 4.0
P.E. (mgm.).....	37	49	39	49	61	50
σ (mgm.).....	55 ± 2.0	73 ± 2.7	58 ± 2.2	73 ± 2.7	91 ± 2.4	75 ± 2.7
C.V. (per cent)....	12.8 ± 0.48	17.0 ± 0.65	18.7 ± 0.72	20.8 ± 0.80	26.0 ± 1.08	28.8 ± 1.15

GENERAL DISCUSSION

It is seen from the results reported herein that plants grown in culture solutions exhibit considerable variability. Indeed, this is of such magnitude as to cast serious doubt upon the practice of drawing conclusions from the means of relatively few cultures. Whenever a worker concerns himself with material of so varying a nature as living organisms and seeks to compare the effect of differing environments, he must first of all ascertain the variation expressed by that material under the condition of the experiment. That such variations cannot be determined with any reasonable degree of accuracy with few variants is patent, and, because of this fact, the average of duplicates can have little meaning unless relatively large differences are being considered. Moreover, arithmetic means of even large numbers of determina-

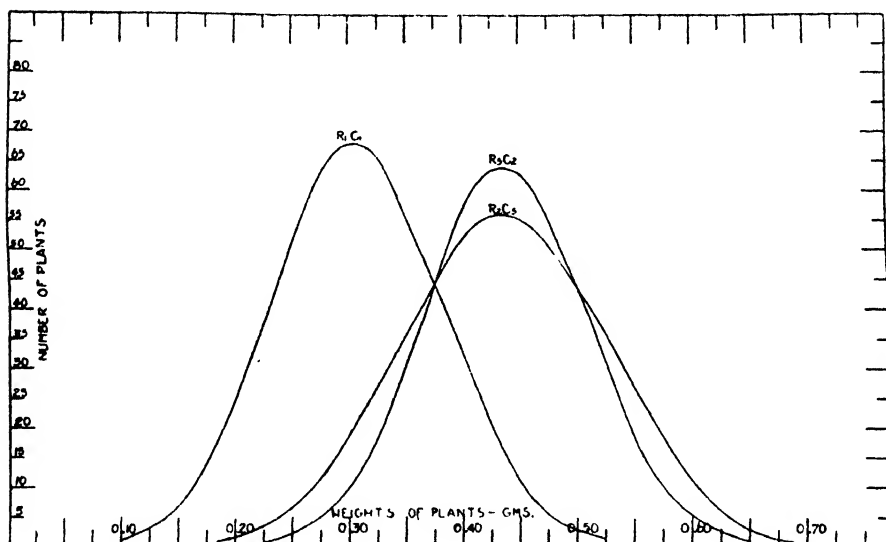


FIG. 11. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

tions of this sort cannot be considered as having fixed value; they are from the very nature of the case only accurate within certain limits, i.e., within their probable error. Until this error of probability is known, it is impossible to place absolute reliance upon the mean itself, no matter how many determinations are averaged. False values may be assigned them, and the conclusions formed be misleading.

In the culture solutions under discussion here, it is apparent that in neither series A, nor B, can a real difference between R_3C_2 and R_2C_3 be demonstrated, whereas had duplicate cultures been employed, the influence of variability upon their means could not have been determined, and solutions might have been given credit for differences due to variability. Thus in series B where

appreciable differences exist between the means of these two solutions, it would have been evidently misleading to have assigned definite values to the two arithmetic means. In Shive's (7) study, there are a number of solutions, such as R_2C_6 , which lie outside the high or low-yield areas as mapped out on his triangles; in fact approximately half of the total number of solutions considered are found within such limits. It is to be wished that our limitations of time and facilities were not such as to make impossible the consideration of all such "medium" solutions, since it is felt that many of them, when interpreted statistically, would show no difference from those making up the high-yield area. Certainly the data reported here would indicate that the designation of a "best" solution, even for a given set of conditions is

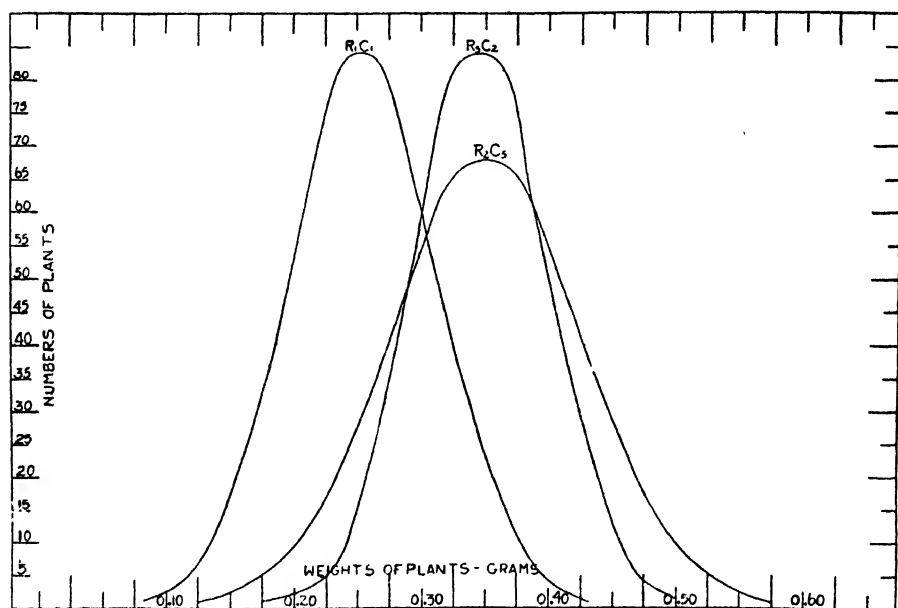


FIG. 12. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

open to doubt. In fact it would seem that, as far as dry yield is concerned, the plant may respond equally well to a number of solutions having relatively wide limits in both total and partial concentrations of salts. In this connection, Stiles (11) noted that where total salt concentrations of 1, 1/5, 1/10, and 1/20 normal were compared, the probable error of the mean dry yield of 10 plants was of such magnitude as to nullify any differences shown by the means alone of the first three solutions.

In mapping out "high" and "low" yield areas, Tottingham (13), Shive (7, 8), and McCall (5) have set rather arbitrary limits. Thus in Shive's first paper (7), as well as in a later (8), the range between solutions giving highest and lowest values is divided into four parts, the upper one-fourth constituting

the high-yield area. In the first investigation, where yields of tops are compared, this high-yield area included but two solutions. From the data given herein, it is evident that, because of variability, such an arbitrary limit fails to give the true boundaries of the area in question. "High-yield areas" are really those made up of solutions giving yields which are not significantly different from those of the most efficient solutions. Thus, if we take R_5C_2 as such a standard, then those solutions would be excluded from the high-yield area whose mean yields give a difference from this standard large enough to be legitimately attributed to something other than normal variation. This would probably mean an enlargement of such areas, since undoubtedly certain "medium" solutions such as R_2C_8 would now have to be included.

McCall (5) in a study similar to Shive's (7) but in which sand was employed instead of water cultures, placed R_5C_2 in the low-yield area, while R_2C_7 , a solution quite markedly different in salt proportions, gave the highest yield. Shive (8), on the other hand, in testing out the effect of the relation of moisture in sand cultures to salt balance, again found R_5C_2 the "best" solution. Wherein rests the explanation for these two widely divergent results? The first thought is of the conditions under which the two experiments were conducted. Shive (9), investigating the influence of sand on a nutrient solution, concluded that if the sand is carefully washed, no material alterations in the physiological properties of the nutrient solutions is to be expected, especially if they are frequently renewed. Both Shive and McCall employed washed sand. It is assumed that such conditions for these studies were not exactly the same, and yet they could hardly have varied sufficiently to be held responsible for the large difference noted. Does it not indicate rather that variability is the causal factor, and that in making comparisons on the basis of duplicate cultures, as has been done here, one is actually comparing fluctuating values? How is one to know whether the relative value assigned to R_5C_2 in Shive's work or in McCall's investigations, reveals the true efficiency of this solution? Obviously the only way we can be certain of comparative values in a study of this sort is to treat the solutions statistically and this can be done only by considering sufficient cultures to give a mean of fair degree of accuracy. This would, of course, preclude the consideration of large numbers of solutions differing slightly in salt proportions, but since it is apparent that small differences in partial salt concentration have little physiological significance, it would seem better to consider few such modifications, using relatively large numbers of replicates for each rather than many modifications employing duplicates only. Conclusions based on the former would have the value of being fairly reliable, while those based upon the latter might be entirely misleading.

In the comparison of culture solutions the importance of using seed of known parentage can hardly be overemphasized. Some varieties of wheat, for example, intercross readily under suitable conditions and the effect of this upon the variability of resulting plants may be marked. In this connection

it would be well worth while knowing whether variation is influenced significantly by different atmospheric environments, since it is usually only with difficulty that such conditions can be exactly duplicated. Again, we know nothing regarding the influence of different culture solutions upon variability. In such a series as Shive's, for example, where we find 36 modifications in partial molal concentration of the constituent salts, it would be of value to know whether any of these solutions increase or decrease the tendency of the plants growing in them to vary. It is expected that this point will be brought out in experiments now under way.

Comparison of many of the well known plant-culture solutions have been made from time to time, but it would seem that here a statistical comparison would be necessary in order to determine definitely their relative efficiency. Shive (7) for example, fixed such relative values for the conditions under which he conducted his experiment. It is doubtful, however, whether much reliance can be placed upon these assigned values, even under the limits which the author imposed, since an insufficient number of plants were employed to give a reliable mean. In fact, many of the studies heretofore made on culture solutions, while exceedingly important in their contribution to our knowledge of salt absorption, do not possess the convincing quality they would have if sufficient plants were employed to give a mean of known accuracy. Since plants do vary we must recognize the fact and allow for it in interpretations, otherwise conclusions may be faulty and future work built upon them prove to be wasted effort.

SUMMARY

The study herein reported concerns the variability shown by plants grown in water cultures and under the commonly accepted methods of control.

Three culture solutions were employed, selected from Shive's (7) 1.75-atmosphere series, and characterized by him as "best," "medium," and "poor" as regards growth efficiency. (The designations used by him were R_5C_2 , R_2C_5 , and R_1C_1 , respectively.) They differed only in the partial molal concentration of constituent salts.

Two series were set up, series A in quart "Mason" jars, consisting of 33 replicate cultures of 5 plants each, and series B in 250-cc. bottles, made up of 50 replicate cultures of 6 plants each. Dry weights of complete plants and of tops alone were employed as criteria of variability.

The following general results were obtained:

1. The variation exhibited by plants of both series was of considerable magnitude, the range being approximately 20 per cent on either side of the mean for culture weights, and about 50 per cent when individual plants were compared. When results were plotted in the form of frequency curves and the curves compared, this variation was of such extent as to show considerable overlapping of data for the three solutions in question.

2. The arithmetic means of the "best" and "medium" solutions, series A, were practically the same, and the calculated frequency curves completely overlapped, thus showing no significant difference in the growth efficiency of these two solutions. In series B, an apparent difference was shown between the means of these two solutions, but the probable error was of such magnitude as to prevent assurance that this was due to anything other than normal variation. In both series the "poor" solution gave a mean significantly different from the other two solutions, thus attesting its real inferiority.

3. No difference was apparent between dry weights of the complete plant and tops alone as criteria of comparison.

4. A chance selection of duplicate cultures from series A gave means varying widely in value. Thus where the mean of the series was 2.15 gm., means of 16 pairs selected at random ranged from 1.82 to 2.55 gm.

It may be concluded that:

1. In comparing the relative growth efficiency of various culture solutions, the limits of accuracy of the means compared must be fixed, i.e., their probable errors must be ascertained, otherwise differences due to variation may be attributed to differences in the efficiency of the solution.

2. The mean of few cultures can have at best but low accuracy; it may vary greatly from the more nearly true mean of a larger series. Such a mean is valueless unless large differences are being compared.

3. There is no one "best" solution, at least under the conditions of control possible to maintain at present; undoubtedly the range of solutions favorable to plant growth is relatively extensive.

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A NEW CLASSIFICATION OF THE SOIL MOISTURE

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INTRODUCTION

The water in the soil is now generally divided into three forms: gravitational, capillary and hygroscopic. The gravitational water is defined as that which is in excess of the amount the soil can retain and which can, therefore, be drawn away by the force of gravity. The capillary water is that part retained in the capillary spaces of the soil and is capable of movement through capillary action. The hygroscopic moisture is the thin film on the surface of the soil particles and is not capable of movement through gravitational or capillary forces. This classification is based on the old idea that the soil is a static framework of solid particles varying in shapes and sizes over the surfaces of which the water spreads as a film and remaining unaffected by the soil and functions as a free liquid. This conception is well reflected by the attempts that have been made to measure the movement and average thickness of the films at definite moisture content, to extract this film of water, to calculate the specific surface of the soil from the size of its particles, etc.

With our present recognition of the colloidal and absorptive properties of the soil, however, we can no longer properly regard the soil as a simple, inactive mass of particles with no influence of its own upon the water. It is now generally admitted that colloidal material does exist in the soil derived both from the humus and clay fractions. This colloidal material is probably responsible for the major portion of the activity of the soil, exerts a controlling influence on the water relationships and makes these relationships between soil and water most intimate and complex.

The present classification of the soil moisture is probably serviceable for practical purposes, but is too general and empirical, and fails to reveal the true facts.

It is the purpose of this paper to present a new classification of the soil moisture which is founded upon a scientific basis, giving the true condition of the moisture in the soil and revealing the intimate and complex relationships that exist between soil and water. According to this new classification the soil water which has heretofore been classed as capillary does not exist in one form but in three different forms: free, capillary-adsorbed and combined.

Before this new classification is presented it will be profitable and advantageous to present first some data revealing the intimate and complex relationships that exist between soil and water and thus to show that the assumptions of the old classification are not correct and that a truer classification is needed.

THE COMPLEX RELATIONSHIPS EXISTING BETWEEN SOIL AND WATER

A few years ago we conceived the idea of trying to measure the concentration of the soil solution directly in the soil by means of the freezing-point method (4). This method has proved very successful and very unique for this purpose. We can now determine the concentration of the soil solution from any maximum to a very low moisture content directly in the soil. The results obtained by this method revealed the intimate and intricate relationships existing between soil and water most clearly and forcibly. It was shown, for instance, that in coarse-textured and uncolloidal soils such as quartz sand and extreme types of sand, the concentration of the soil solution increased

TABLE 1
Lowering of the freezing point of quartz sand at various moisture contents

MOISTURE	OBSERVED LOWERING OF THE FREEZING POINT	CONSTANT K
<i>per cent</i>	<i>°C.</i>	
2	0.091	0.182
6	0.027	0.162
10	0.018	0.180
14	0.012	0.168
18	0.009	0.162

inversely proportionally to the moisture content, and could be expressed by the simple equation $MD = K$, where K is the resultant constant, M the percentage of moisture and D the observed depression of the freezing point. In the fine-textured and colloidal soils, on the other hand, the concentration of the soil solution increased in a geometric progression as the water content decreased in an arithmetic progression and could be mathematically expressed by the equation $D = AR^{n-1}$ where D is the freezing-point depression, A the first depression, R the ratio of any depression (except the first) to the preceding one, and N the number of the depression. Typical examples of these two sets of results are shown in tables 1 and 2.

It is readily seen, therefore, that in the case of the quartz sand the concentration increases in a direct ratio with the decrease of the moisture content, while in the case of the soil it increases at an abnormally greater rate than the moisture decreases.

The freezing-point method revealed the intimate and complex relationships that exist between soil and water still in another way. It was found that in all classes of soil, with the exception of sands, the magnitude of the freezing-

TABLE 2

Lowering of the freezing point of clay loam at various moisture contents

MOISTURE	OBSERVED LOWERING OF THE FREEZING POINT	CALCULATED LOWERING OF THE FREEZING POINT
<i>per cent</i>	°C.	
10.0	1.292	
12.5	0.612	0.956
15.0	0.377	0.453
17.5	0.252	0.279
20.0	0.162	0.186
22.5	0.112	0.120
25.0	0.082	0.083
27.5	0.050	0.060
30.0	0.037	0.037

TABLE 3

Effect of repeated freezing and thawing upon the lowering of the freezing point

MOISTURE	NUMBER OF TIMES FROZEN	LOWERING OF THE FREEZING POINT
Sandy loam		
<i>per cent</i>		°C.
4	1	0.380
	2	0.340
	3	0.330
Clay loam		
15	1	0.820
	2	0.530
	3	0.520
	4	0.515
Silt loam		
16	1	0.430
	2	0.280
	3	0.220
	4	0.220
Clay		
18	1	0.870
	2	0.600
	3	0.470
	4	0.465

point lowering at a low moisture content, decreased with successive freezing and thawing, and the magnitude of this diminution tended to increase with the fine texture and colloidal content of the soil. Typical examples of these results are shown in table 3.

These data show most strikingly and unmistakably, therefore, that the process of repeated freezing and thawing tends to reduce the initial freezing-point lowering considerably.

For an explanation of these phenomena as well as those showing that the freezing-point depression increases at an abnormally greater rate than the moisture content decreases, the following hypotheses were offered:

(a) The soils have the ability to cause a certain amount of water to become unfree. This unfree water may be in the soil either as capillary-adsorbed or chemically combined or both. In either event this unfree water is not free or active to function as a solvent but is removed from the active liquid phase and also from the field of action as far as the freezing-point lowering is concerned. Thus, if a clay causes 15 per cent of water to become unfree, and at 39 per cent of moisture this clay gives a depression of 0.075°C . and at 22 per cent 0.987° , then in the first case there is 24 per cent of moisture free or active to dissolve the salts, while in the second case there is only 7 per cent of free or active water for the same purpose. The depression of the freezing-point lowering at the low moisture content, therefore, would be many times greater than at the high, than would be expected from the total percentage of water.

(b) The water which the soils cause to become unfree and thus removed from the field of action as far as the freezing-point lowering is concerned, is due to the colloids which the soils contain and to the capillarities of the soil. A portion of the unfree water exists in the colloids as physically adsorbed and loosely chemically combined (3) and another portion as unfree capillary water in the capillarities of the soil. Upon freezing the colloids are coagulated, the bonds uniting them with the water break and some of the unfree water becomes liberated. The capillarities of the soil are also destroyed by the process of freezing and the unfree capillary water also becomes liberated. This liberated and now free water from both sources goes to dilute the original soil solution and thereby decreases the original lowering of the freezing point.

The results yielded by the freezing-point method together with the hypotheses advanced to explain them and the conclusions derived therefrom were confirmed completely and in a most remarkable way by the dilatometer method (2). In brief, this method has shown absolutely: (a) that soils do cause water to become unfree, (b) that the process of repeated freezing and thawing liberates some of this unfree water and (c) that the moisture which has heretofore been classed as capillary does not exist all in one form but can be divided into three different forms: free, capillary-adsorbed and combined.

The principle of the dilatometer method is based upon the fact that water expands upon freezing. Knowing the coefficient of expansion of water upon freezing and also the total water content of a soil, then the amount of water that freezes and does not freeze can be easily calculated.

The general procedure of the method consists of adding a definite amount of soil and water or a soil of known moisture content into the dilatometer,

filling the empty space with ligroin and then causing the moisture to freeze at different degrees of supercooling.¹

It was found by this method that part of the moisture of a wet soil freezes very readily at slightly below $0^{\circ}\text{C}.$, another portion does not freeze until a temperature of $-4^{\circ}\text{C}.$ was reached, and a third portion does not freeze at any temperature—not even down to -78° . In many clays and clay loams as much as 40 per cent of the water added failed to freeze even at the extreme low temperature of -78° , while in quartz sand and sands all the water added froze readily at slightly below 0° .

NEW CLASSIFICATION OF THE SOIL MOISTURE

Most obviously, therefore, the moisture in the heavier classes of soil above the hygroscopic point does not all exist in one form. If it all existed in one form it certainly should have all frozen at one temperature and at the temperature of slightly below 0° as was the case of the moisture in the quartz sand and sands. Since it required different degrees of cooling to freeze, and some did not freeze at all, then there seems to be a strong proof that the moisture which has heretofore been classed as capillary exists in more than one form in all the agricultural soils with the exception of the plain sands and some coarse sandy loams containing no organic matter. On the basis of the dilatometer method it would appear reasonable and logical to consider that portion of the water which freezes readily at slightly below 0° to be different from that which freezes at -4° and $-78^{\circ}\text{C}.$, and this to be different from that which does not freeze even at the extreme lower temperature. Accordingly, therefore, the moisture of the soils should be classified into the following forms on the basis of the dilatometer method:

Gravitational	
Free	
Unfree	$\left\{ \begin{array}{l} \text{capillary-adsorbed} \\ \text{combined} \left\{ \begin{array}{l} \text{water of solid solution or} \\ \text{water of hydration.} \end{array} \right. \end{array} \right.$

The gravitational water needs no explanation of course. The free water is that which freezes for the first time at the supercooling of $-1.5^{\circ}\text{C}.$ The capillary-adsorbed water is that which freezes finally at the supercooling of -4° and also in the temperature of -78° minus the free water. The combined water is that which does not freeze at all.

The temperature of -1.5° has been chosen for the free water first because the freezing point of the soil is not 0° but somewhat less than 0° , and second because the rate of freezing near 0° is very slow. The temperature of -4° is chosen for the capillary-adsorbed water, first because this is the greatest degree of supercooling that the soil will withstand without premature solidification,¹

¹ For detailed description of the method and procedure see (2).

and second because at this temperature nearly all the water that is freezable will freeze. This is shown by the fact that very little if any additional water freezes at -78° which does not freeze at -4° .

It appears logical to call all the water which freezes near 0° free water because pure water in mass freezes at 0° . Water which does not freeze at this temperature must be different from free water. Since the physical condition of the soil presupposes that some of its water must exist around and in the interstices of its particles, and that this water probably has a lower vapor pressure corresponding to a lower freezing point, it appears reasonable to call this water capillary-adsorbed. Water on the other hand, which does not freeze at all even at the extreme low temperature of -78° must also be different from the capillary-adsorbed water. Since it is known that certain solid materials contain water of hydration, solid solution of water, etc., it has seemed reasonable to call this water which does not freeze combined.

RELATIVE AMOUNTS OF THE DIFFERENT FORMS OF WATER

The ability of the dilatometer method to distinguish and classify the moisture within any soil into the various forms between the various types of soil is revealed in tables 4 and 5. In these tables are presented the results of a very comprehensive list of soils representing all classes and many types. In table 4 the relative amounts of the different forms of water are based only on the water added, while in table 5 they are based both on the water added and also on the hygroscopic moisture.

The data in these tables reveal many facts of great interest. They show first of all that water in the soils does exist in different forms and that the amount of these different forms varies tremendously in the various soils. In some soils only one or two forms predominate, while in others all three are about equally divided. In sands and fine sandy loams it is the free water that predominates, which amounts in many cases to about 95 per cent of the total water present; the other 5 per cent consists as a rule of combined water; capillary-adsorbed water is apparently not common in most of these classes of soil. In loams and silt loams practically the same conditions hold except that more water is present in the combined form. In the clay loams, humus loams and clays it is the combined water that predominates followed in order by capillary adsorbed and free water, respectively.

SIGNIFICANCE OF THE NEW CLASSIFICATION OF SOIL MOISTURE

The dilatometer method not only affords us a true and scientific basis for the classification of the soil moisture into its proper forms but also gives us a deeper and truer insight regarding the behavior and functions of the soil moisture and its bearing upon other phenomena in the soil, such as the movement of moisture, evaporation, wilting coefficient, physiologically unavailable

TABLE 4

Relative amounts of the different forms of water based on the amount of water added

NUM- BER	SOILS	FREE WATER		CAPILLARY- ADSORBED WATER		COMBINED WATER
		cc.	per cent	cc.	per cent	per cent
1	Quartz sand	5.0	100.0	0	0	0
2	Coarse sand	4.80	96.0	0	0	4.0
3	Medium fine sand	4.70	94.0	0	0	6.0
4	Illinois medium fine sand	4.70	94.0	0	0	6.0
5	Wisconsin Plainfield fine sand	4.45	89.0	0	0	11.0
6	Fine sandy loam	4.20	84.0	0	0	16.0
7	California Yolo fine sandy loam	4.20	84.0	0.20	4.0	12.0
8	California Harford fine sandy loam	4.25	85.0	0.15	3.0	12.0
9	Kentucky LaCrosse sandy loam	4.00	80.0	0.35	7.0	13.0
10	Illinois White silt loam	4.15	83.0	0.25	5.0	12.0
11	Kentucky Miami silt loam	4.05	81.0	0.05	1.0	18.0
12	Holland loam	3.40	68.0	0.70	14.0	18.0
13	Illinois Brown silt loam	3.40	68.0	0.50	10.0	22.0
14	Wisconsin Colby silt loam	3.50	70.0	0.20	4.0	26.0
15	Illinois brown silt loam	3.25	65.0	0.65	13.0	22.0
16	Wisconsin Carrington silt loam	3.05	61.0	0.25	5.0	34.0
17	Kentucky Carrington loam	3.05	61.0	0.15	3.0	36.0
18	Heavy brown silt loam	2.70	54.0	0.55	11.0	35.0
19	Heavy dark brown silt loam	1.70	34.0	1.50	30.0	36.0
20	Heavy dark brown silt loam	1.85	31.0	1.20	24.0	39.0
21	Kentucky Marshall silt loam	2.25	45.0	1.05	21.0	34.0
22	California Romona clay loam	2.30	46.0	1.00	20.0	34.0
23	Illinois black clay loam	1.70	34.0	1.50	30.0	36.0
24	California Chino silty clay loam	1.00	20.0	1.75	35.0	45.0
25	Kentucky Carrington clay loam					
26	Wisconsin Superior clay	1.60	32.0	1.50	30.0	38.0
27	Clay	1.20	24.0	1.80	36.0	40.0
28	Minnesota Superior clay	0.80	16.0	2.45	49.0	35.0
29	Norfolk sand, Coffee County, Ala.	4.50	90.0	0	0	10.0
30	Norfolk sand, Anne Arundel Co., Ind	4.70	94.0	0	0	6.0
31	Norfolk sand, Johnson County, N. C.	4.65	93.0	0	0	7.0
32	Dekalb sandy loam, Blount County, Ala.	4.50	90.0	0	0	10.0
33	Dekalb sandy loam, Etowah County, Ala.	4.35	87.0	0	0	13.0
34	Dekalb sandy loam, Blount County, Ala.	4.30	86.0	0	0	14.0
35	Norfolk sandy loam, Jones County, Ga.	4.65	93.0	0	0	7.0
36	Norfolk sandy loam, Bamberg County, S. C.	4.70	94.0	0	0	6.0
37	Norfolk sandy loam, Milles County, Ga.	4.50	90.0	0	0	10.0
38	Norfolk fine sandy loam, Wayne County, Miss.	4.40	88.0	0	0	12.0
39	Norfolk fine sandy loam, Bamberg County, S. C.	4.00	92.0	0	0	8.0
40	Norfolk fine sandy loam, Winston County, Miss.	4.50	90.0	0	0	10.0

TABLE 4—*Concluded*

NUM- BER	SOILS	FREE WATER		CAPILLARY- ADSORBED WATER		COMBINED WATER
		cc.	per cent	cc.	per cent	per cent
41	Vernon fine sandy loam, Archer County, Texas.....	3.55	71.0	0.55	11.0	18.0
42	Vernon fine sandy loam, Archer County, Texas.....	3.70	74.0	0.60	12.0	14.0
43	Vernon fine sandy loam, Taylor County, Texas.....	3.85	77.0	0.15	3.0	20.0
44	Hagerstown loam, Polk County, Ga.....	3.80	76.0	0.45	9.0	15.0
45	Hagerstown loam, Lawrence County, Ala..	3.55	71.0	0.35	7.0	22.0
46	Hagerstown loam, Madison County, Ala..	3.95	79.0	0.10	2.0	19.0
47	Carrington loam, Barnes County, N. D...	2.20	44.0	0.80	16.0	40.0
48	Carrington loam, Goodline County, Minn.	1.60	32.0	1.90	38.0	30.0
49	Carrington loam, Sioux County, Ia.....	3.20	64.0	0.60	12.0	24.0
50	Summit silt loam, Cass County, Mo.....	2.40	48.0	1.15	23.0	29.0
51	Summit silt loam, Barton County, Mo....	3.20	64.0	0.60	12.0	24.0
52	Miami silt loam, Delaware County, Ind...	3.60	72.0	0.60	12.0	16.0
53	Miami silt loam, Hendricks County, Ind..	3.75	75.0	0.65	13.0	12.0
54	Miami silt loam, Boone County, Ind.....					
55	Memphis silt loam, Wilkinson County, Miss.....	2.45	49.0	1.10	22.0	29.0
56	Memphis silt loam, Wilkinson County, Miss.....	4.05	81.0	0.25	5.0	14.0
57	Marshall silt loam, Sioux County, Ia....	2.40	48.0	1.00	20.0	32.0
58	Marshall silt loam, Goodline County, Minn.....	1.85	37.0	1.70	34.0	29.0
59	Marshall silt loam, Nodaway County, Mo.	2.40	48.0	1.15	23.0	29.0
60	Kirkland silt loam, Payne County, Okla...	2.95	59.0	1.00	20.0	21.0
61	Kirkland silt loam, Payne County, Okla..	3.25	65.0	0.75	15.0	20.0
62	Vernon clay loam, Kay County, Okla.....	2.65	53.0	0.60	12.0	35.0
63	Vernon clay loam, Archer County, Texas..	3.70	74.0	0.30	6.0	20.0
64	Vernon clay loam, Roger Mills County, Okla.....	3.25	65.0	0.65	13.0	22.0
65	Lufkin clay, Columbia County, Ark.....	2.50	50.0	1.10	22.0	28.0
66	Lufkin clay, Caddo Parish, La.....	3.50	70.0	0.50	10.0	20.0
67	Lufkin clay, Winston County, Miss.....	1.00	20.0	2.65	53.0	27.0
68	Houston clay, Franklin County, Texas....	0.75	15.0	2.25	45.0	40.0
69	Houston clay, Grayson County, Texas....	1.85	37.0	1.80	36.0	27.0
70	Houston clay, Ellis County, Texas.....	2.70	54.0	1.00	20.0	26.0
71	Cecil clay, Troup County, Ga.....	1.65	33.0	1.35	27.0	40.0
72	Cecil clay, Jackson County, La.....	0.80	16.0	2.45	49.0	35.0
73	Cecil clay, Randolph County, N. C.....	1.55	31.0	1.50	30.0	39.0

TABLE 5

Relative amounts of the different forms of water, based on the amount of water added and also on the hygroscopic water

NUM- BER	SOILS	FREE WATER		CAPILLARY- ADSORBED WATER		COMBINED WATER
		cc.	per cent	cc.	per cent	per cent
1	Quartz sand.....	5.0	100.00	0	0	0
2	Coarse sand.....	4.80	94.12	0	0	5.88
3	Medium fine sand.....	4.70	91.10	0	0	8.90
4	Illinois medium fine sand.....	4.70	91.10	0	0	8.90
5	Wisconsin Plainfield fine sand.....	4.45	85.81	0	0	14.19
6	Fine sandy loam.....	4.20	80.00	0	0	20.00
7	California Yolo fine sandy loam.....	4.20	79.56	0.20	3.78	16.66
8	California Harford fine sandy loam.....	4.25	78.70	0.15	2.77	18.53
9	Kentucky La Crosse sandy loam.....	4.00	75.19	0.35	6.57	18.24
10	Illinois white silt loam.....	4.15	78.16	0.25	4.71	17.13
11	Kentucky Miami silt loam.....	4.05	75.70	0.05	0.93	23.37
12	California Holland loam.....	3.40	63.09	0.70	12.99	23.92
13	Illinois brown silt loam.....	3.40	59.66	0.50	8.77	31.57
14	Wisconsin Colby silt loam.....	3.50	61.40	0.20	3.52	35.08
15	Illinois brown silt loam.....	3.25	57.01	0.50	8.77	34.22
16	Wisconsin Carrington silt loam.....	3.05	53.50	0.25	4.38	42.12
17	Kentucky Carrington loam.....	3.05	51.26	0.15	1.93	46.81
18	Heavy brown silt loam.....	2.70	46.55	0.55	9.48	43.97
19	Heavy dark brown silt loam.....	1.70	29.56	1.50	26.09	43.35
20	Heavy dark brown silt loam.....	1.85	31.10	1.20	20.17	48.73
21	Kentucky Marshall silt loam.....	2.25	39.48	1.05	18.42	41.10
22	California Romona clay loam.....	2.30	38.34	1.00	16.67	44.99
23	Illinois black clay loam.....	1.70	16.70	1.50	23.55	49.75
24	California Chino silty clay loam.....	1.00	15.80	1.75	27.65	56.55
25	Kentucky Carrington clay loam.....					
26	Wisconsin Superior clay.....	1.60	26.40	1.50	24.75	48.85
27	Clay.....	1.20	19.80	1.80	29.70	50.50
28	Minnesota Superior clay.....	0.80	12.61	2.28	36.00	51.36
29	Norfolk sand, Coffee County, Ala.....	4.50	89.23	0	0	10.77
30	Norfolk sand, Anne Arundel County, Ind..	4.70	93.26	0	0	6.74
31	Norfolk sand, Johnson County, N. C.....	4.65	92.28	0	0	7.72
32	Dekalb sandy loam, Blount County, Ala..	4.50	88.24	0	0	11.76
33	Dekalb sandy loam, Etowah County, Ala..	4.35	85.30	0	0	14.70
34	Dekalb sandy loam, Blount County, Ala..	4.30	84.30	0	0	15.70
35	Norfolk sandy loam, Jones County, Ga....	4.65	91.54	0	0	8.46
36	Norfolk sandy loam, Bamberg County, S. C.	4.70	92.52	0	0	7.48
37	Norfolk sandy loam, Milles County, Ga....	4.50	88.60	0	0	11.40
38	Norfolk fine sandy loam, Wayne County, Miss.....	4.40	84.94	0	0	15.06
39	Norfolk fine sandy loam, Bamberg County, S. C.....	4.60	88.80	0	0	11.20
40	Norfolk fine sandy loam, Winston County, Miss.....	4.50	86.88	0	0	13.12

TABLE 5—*Concluded*

NUM- BER	SOILS	FREE WATER		CAPILLARY- ADSORBED WATER		COMBINED WATER
		cc.	per cent	cc.	per cent	per cent
41	Vernon fine sandy loam, Archer County, Texas.....	3.55	65.14	0.55	10.09	24.77
42	Vernon fine sandy loam, Archer County, Texas.....	3.70	67.90	0.60	11.01	21.09
43	Vernon fine sandy loam, Taylor County, Texas.....	3.85	70.64	0.15	2.11	27.25
44	Hagerstown loam, Polk County, Ga.....	3.80	70.00	0.45	8.20	21.80
45	Hagerstown loam, Lawrence County, Ala..	3.55	65.40	0.35	6.44	28.16
46	Hagerstown loam, Madison County, Ala..	3.95	72.75	0.10	1.84	25.41
47	Carrington loam, Barnes County, N. D. . .	2.20	39.43	0.80	14.36	46.21
48	Carrington loam, Goodline County, Minn..	1.60	28.68	1.90	34.05	37.27
49	Carrington loam, Sioux County, Ia.	3.20	57.35	0.60	10.75	31.90
50	Summit silt loam, Cass County, Mo.	2.40	43.17	1.15	20.68	36.18
51	Summit silt loam, Barton County, Mo. . .	3.20	57.55	0.60	10.77	31.68
52	Miami silt loam, Delaware County, Ind. .	3.60	67.42	0.60	11.24	21.34
53	Miami silt loam, Hendricks County, Ind..	3.75	70.22	0.65	12.17	17.61
54	Miami silt loam, Boone County, Ind.					
55	Memphis silt loam, Wilkinson County, Miss.....	2.45	42.62	1.10	19.13	38.25
56	Memphis silt loam, Wilkinson County, Miss.....	4.05	70.43	0.25	4.35	25.22
57	Marshall silt loam, Sioux County, Ia.	2.40	41.17	1.00	17.16	41.67
58	Marshall silt loam, Goodline County, Minn..	1.85	31.73	1.70	29.16	39.11
59	Marshall silt loam, Nodaway County, Mo	2.40	41.17	1.15	19.73	39.10
60	Kirkland silt loam, Payne County, Okla. .	2.95	51.76	1.00	17.55	30.69
61	Kirkland silt loam, Payne County, Okla. .	3.25	57.02	0.75	13.16	29.82
62	Vernon clay loam, Kay County, Okla. . . .	2.65	44.85	0.60	10.15	45.00
63	Vernon clay loam, Archer County, Texas .	3.70	62.60	0.30	5.07	32.33
64	Vernon clay loam, Roger Mills County, Okla.....	3.25	55.00	0.65	11.00	34.00
65	Lufkin clay, Columbia County, Ark.	2.50	46.00	1.10	20.26	33.74
66	Lufkin clay, Caddo Parish, La.	3.50	64.46	0.50	9.21	26.33
67	Lufkin clay, Winston County, Texas. . . .	1.00	18.42	2.65	48.80	32.78
68	Houston clay, Franklin County, Texas. . .	0.75	12.50	2.25	37.50	50.00
69	Houston clay, Grayson County, Texas. . .	1.85	30.83	1.80	30.00	39.17
70	Houston clay, Ellis County, Texas.	2.70	4.50	1.00	16.67	38.33
71	Cecil clay, Troup County, Ga.	1.65	29.68	1.35	24.28	46.04
72	Cecil clay, Jackson County, La.	0.80	14.39	2.45	44.05	41.56
73	Cecil clay, Randolph County, N. C.	1.55	27.88	1.50	26.98	45.14

water, etc. Indeed with a knowledge of this new classification we are enabled not only to understand but also to predict what to expect regarding these phenomena. Thus for instance, in regard to the movement of moisture it could be safely predicted that a large percentage of water in the fine-textured or colloidal soils is immovable as far as capillary movement is concerned. This conclusion would be self-evident and justifiable from the fact that a large amount of water is not free to freeze in these soils even at the extreme low temperature of $-78^{\circ}\text{C}.$, and if it is not free to freeze at this low temperature it is certainly not free to move capillaryly.

As regards the rate of evaporation, it could be predicted and expected that the rate would be different at the various moisture contents. The free water would possess one velocity of evaporation, the capillary-adsorbed another and the combined still another. The free water would possess the greatest velocity of evaporation, the combined the smallest and the capillary-adsorbed an intermediate.

The phenomenon of the wilting coefficient of soils becomes also more intelligible in the light of the dilatometer results. The consensus of opinion among soils men and plant physiologists is that the plants wilt even when there is still plenty of moisture in the soil, because the movement of the moisture to the roots of the plants is not sufficiently rapid to supply the water lost by transportation. This consensus of opinion is well expressed by Shull (8). He says:

The wilting of plants at the wilting coefficient of the soil can not be due to lack of moisture in the soil nor to lack of a gradient of forces tending to move water toward the plant. The view is held, therefore, that the wilting at this critical soil-moisture content must be due to the increasing slowness of water movement from soil particle to soil particle and from these to the root hairs, the rate of movement falling below that necessary to maintain turgidity of the cells of the aerial parts, even under conditions of low transportation.

In the light of the dilatometer results, however, the plants wilt not because the soil moisture does not move at a sufficiently rapid rate but because it does not move at all. As has already been seen the capillary-adsorbed water freezes with great difficulty and the combined not at all. Now if the soil moisture is not free to freeze it is certainly not free to move capillaryly.

The more correct reasons for the wilting of plants at the wilting coefficient of soils appears to be the following: (a) The moisture near the wilting coefficient is held by the soil with such force that the plants can not extract it. A large part of this water is probably not in the liquid state. (b) Near the wilting coefficient the concentration of the soil solution is comparatively high which would tend to influence the intake of water by the roots. At the moisture content where there is no more free water but only capillary-adsorbed and combined the concentrations of the soil solutions, as proved experimentally (4), is greater than that of the cell sap of the roots.

The new physical classification of the soil moisture on the basis of the dilatometer results enables us now to classify the soil moisture also on a true

and scientific physiological basis. The old physiological classification of the soil moisture consists of dividing the soil water broadly into available and unavailable. From a practical standpoint this classification is probably serviceable but it is too general, empirical and does not give a true, detailed and definite information regarding the availability and unavailability of the different forms of water. The new classification, however, which is suggested by the results of the dilatometer method and supported by physiological studies seems to meet all these requirements. This new physiological classification of the soil moisture is as follows:

Gravitational	{ unsuitable or superavailable	
Free	{ readily available	
	{ capillary-adsorbed	{ very slightly available
Unfree	{ combined	{ water of hydration
		{ water of solid solution
		{ unavailable

According to this classification it is only the free water or the water which freezes at the supercooling of about -1.5°C . that the plants can take up very readily. For this water the plant exerts very little, if any, force to utilize it because it exists in a free condition and is not held very rigidly by any outside forces. It is this water with which the plant makes its growth. After the plant uses up this free water it generally begins to wilt.

The capillary-adsorbed water, or the water which freezes at the supercooling of -4° and in the temperature of -78° is available to the plant only slightly and under certain conditions. For this water the plant has to exert force to obtain it, because it is held by the soil with considerable force. With this water the plant is probably not able to make growth but simply to sustain life.

The combined water, or the water which does not freeze at all, even at the extreme low temperature of -78° , is ordinarily not at all available to the plant. This water probably exists in the solid phase and is held by the soil with tremendous forces.

This new physiological classification of the soil moisture appears to be amply supported by the large amount of data obtained on the wilting of plants and by a direct comparison made between the wilting coefficient and the different forms of water. The work of Briggs and Shantz (5) shows that plants wilt when the total soil moisture is still high which is considerably above the combined water. A direct comparison made between the wilting coefficient of some of these soils employed by Briggs and Shantz and the dilatometer method results show that the percentage of moisture which fails to freeze at the supercooling of -1.5°C . is very closely the same as that at which plants begin to wilt, indicating that the wilting coefficient of soils is at the point where the free moisture ends and the capillary-adsorbed moisture begins. On the other hand, the extensive investigations of Alway (1) in which he allowed the plants to grow almost to maturity show that the plants are able to reduce

the soil moisture down to the hygroscopic coefficient. Now this hygroscopic coefficient seems to represent about the same degree of moisture as the combined water, indicating, therefore, that the capillary-adsorbed water is available to plants under certain conditions.

In view of the close agreement that appears to exist between the wilting coefficient and the unfree water as determined by the dilatometer method, it would seem logical and advisable, therefore, to determine this factor by the dilatometer method. The percentage of water that fails to freeze for the first time at the supercooling of -1.5°C . can be taken to represent the upper limits of moisture content at which plants may begin to wilt, while the percentage of moisture which fails to freeze at -4°C . can be taken to represent the lower limits at which plants are able at all to extract the moisture from the soil under the most favorable conditions.

The determination of the wilting coefficient of soils by means of the plant, besides being tedious and time consuming, is not accurate nor constant. The investigations of Caldwell (6) and of Shive and Livingston (7) show that the permanent wilting of the plant is a function of the intensity of atmospheric evaporation. By means of the dilatometer method on the other hand, the determination of the wilting coefficient is more definite and more comparable and, of course, infinitely easier, more convenient and rapid.

The new classification of the soil moisture, therefore, is founded on experimental and scientific principles and appears to classify the soil moisture into its actual forms and thus reveals its actual conditions, its intimate and complex relationships with the soils and its bearing upon many phenomena in the soil.

SUMMARY

The object of this paper is to present a new classification of the soil moisture which is founded on experimental and scientific principles and which appears to show the actual condition of the moisture in the soil.

This new classification is based upon the principle of the freezing of water. It is found that a portion of the soil water freezes very readily near 0°C ., another portion freezes only when a temperature of -4° is reached and a third portion does not freeze at all, even at the extreme low temperature of -78°C .

Obviously, the water in the soil above the hygroscopic moisture, can not all be in one form, i.e. capillary, because if it were all in one form all of it ought to freeze at one temperature and indeed near 0° . Since different portions of it freeze at different temperatures or not at all, then it must exist in different conditions.

On the basis of these experimental and scientific facts the soil moisture lends itself to the following classification:

Gravitational	
Free	
Unfree	$\left\{ \begin{array}{l} \text{capillary-adsorbed} \\ \text{combined} \left\{ \begin{array}{l} \text{water of solid solution} \\ \text{water of hydration} \end{array} \right. \end{array} \right.$

The free water is that which freezes for the first time at the supercooling of -1.5°C ., the capillary-adsorbed water is that which freezes finally at the supercooling of -4° and at the cooling of -78° , minus the free water. The combined water is that which does not freeze at all, even at the temperature of -78° .

It appears reasonable and logical to call all water freezing near 0° free water because pure water in mass freezes at 0° . Water which does not freeze at this temperature must be different from free water. Since the physical condition of the soil presupposes that some of its water must exist around and in the interstices of its particles, and that this water probably has a lower vapor pressure corresponding to a lower freezing point, it appears reasonable and logical to call this water capillary-adsorbed. Water on the other hand which does not freeze at all even at the extreme low temperature of -78°C . must also be different from the capillary-adsorbed. Since it is known that certain solid materials contain water of hydration, solid solution of water, etc., it has seemed reasonable to call this water which does not freeze combined water.

On the basis of this classification it was found that in some soils only one or two forms of water exist while in others all three forms exist but in different proportions.

The method that is capable of measuring the relative amounts of these various forms of water in the soil is the dilatometer method. The principle of this method is based upon the fact that water expands upon freezing. If the coefficient of expansion of water is known and also the total percentage of water in the soil, then the amount of water that freezes or fails to freeze can be readily ascertained.

The procedure of the method consists of adding a definite amount of soil and water or a soil of known moisture content, into the dilatometer, filling the empty space with ligroin and then causing the soil moisture to freeze. It is first supercooled to -1.5°C . and the free water allowed to freeze at this temperature. Then the contents of the dilatometer are thawed and frozen at the temperature of -15° for half an hour. Then they are thawed again and supercooled to -4° where the capillary-adsorbed water freezes. When the contents come in equilibrium with the temperature of -4° , they may be cooled to -78°C . and brought back again to the temperature of -4° . It is not always necessary, however, to cool the soil to -78° because practically all the water that is freezable will freeze at -4° .

It is found that repeated freezing and thawing causes some of the unfree water to become free. This unfree water which becomes free belongs entirely to the capillary-adsorbed water.

The new classification of the soil moisture gives a clearer and deeper insight of the actual condition of the soil moisture, its intimate and complex relationships with the soil and its bearing upon many phenomena in the soil such as the movement of moisture, evaporation, wilting coefficient of soils, availability and unavailability of moisture, etc.

In the light of the dilatometer results, together with those of physiological studies, the soil moisture can now be classified more definitely and scientifically, also on a physiological basis. This new physiological classification of the soil moisture is as follows:

Gravitational	{	superavailable	
Free	{	very available	
	{	capillary-adsorbed	{ only slightly available
Unfree	{	combined	{ water of solid solution
			{ water of hydration } unavailable

The dilatometer method is capable of determining also the wilting coefficient of soils, and this determination is more accurate, definite and comparable than that by means of the plant, and of course, infinitely more convenient, easier and rapid.

The old classification of the soil moisture is too empirical and general and does not tell the true story of the actual condition of the soil moisture. It is based on the old idea that the soil is a simple mass of solid particles over the surface of which the moisture spreads and remaining unaffected by the soil itself. The soil, however, is a very complex mass and its relationships with water are very intimate and intricate. This old classification, therefore, must sooner or later give way to a new one which is based upon actual facts, such as the new classification proposed in this paper.

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SULFUR AND SULFUR COMPOSTS IN RELATION TO PLANT NUTRITION

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A few years ago Lipman and co-workers (8) called attention to the possibility of rendering insoluble phosphates available as fertilizers by composting them with sulfur. Data from soil composts were presented which showed that sufficient acid might be produced by the biological oxidation of sulfur to produce soluble phosphates, after the general manner of the manufacture of acid-phosphate. Although the basis of the earlier composting experiments was soil of various types, it seems to have been generally recognized that a liberal supply of organic matter is favorable to the process.

Brown and Warner (3) have secured results like those of Lipman, using manure as the basis of their composts. They found that the addition of sulfur increased the solubility of phosphorus, as compared with untreated, fermented manure. Composting rock-phosphate together with manure and sulfur led to remarkable increases of available phosphorus, as measured by extraction with solution of ammonium citrate. Ames and Richmond (1), working with soil composts, and Shedd (12), with composts of soil and manure, have obtained similar favorable effects of sulfonation upon the availability of rock phosphate.

EXPERIMENTATION

A. Composts

The results reported herewith were obtained by applying Lipman's procedure to the composting of "floats," or finely ground rock-phosphate, with manure or soil. In general, we have followed Lipman's methods closely, so as to make our results comparable with his, but the composts have been handled on a larger scale.

Two types of soil were used.² One was Miami silt loam, manured at the rate of 40 tons per acre; the other was a garden soil, prepared by composting Miami silt loam with manure, leaf mold and sod. Duplicate portions of compost were made as follows:

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² C. D. Williams should receive credit for performing this phase of the investigation.

1. 480 gm. soil.
2. 420 gm. soil and 60 gm. rock-phosphate.
3. 460 gm. soil and 20 gm. sulfur.
4. 400 gm. soil and 20 gm. sulfur and 60 gm. rock-phosphate.

All of these materials were dried by exposure to the air, ground and passed through a 40-mesh sieve, in preparation for use. The well mixed composts were placed in Mason jars of 1 quart capacity. Water was then added to the extent of 50 per cent of the total water-holding capacity, as measured by Hilgard's method (6). Afterward, the jars were weighed, covered loosely and stored at room temperature. At regular intervals the composts were restored to original weights by adding water. After intervals of 4, 8, 12 and 32 weeks they were mixed thoroughly and samples equivalent to 50 gm. of the original air-dried composts were dried by exposure to the air and pulverized for analysis.

The methods of analysis were as follows:

Citrate-soluble P_2O_5 . Five grams of sample were extracted with 100 cc. of neutral solution of ammonium citrate kept at 65°C. for 30 minutes, according to the official method of agricultural chemists (2). An aliquot of the extract equivalent to 0.5 gm. of sample was evaporated after adding HNO_3 and $Mg(NO_3)_2$, and ignited. Final determination was by the volumetric method.

Water-soluble P_2O_5 . Ten grams of sample were shaken with 160 cc. of water, originally boiling, for 30 minutes. Aliquots were treated as in the preceding determination.

SO_3 . Five grams of sample were shaken with 100 cc. of 1.0 per cent HCl for 7 hours. SO_3 was determined by precipitation with $BaCl_2$ in the usual manner.

Total acidity. Extracts prepared as for determining water-soluble P_2O_5 were boiled and titrated with 0.02 *N* NaOH, using phenolphthalein as indicator. The results have been made comparable with those of Lipman by expression as equivalents of 120 gm. of compost.

H^+ concentration. This was determined by the colorimetric method of Gillespie (4), with extracts prepared as for determining total acidity.

The data resulting from these analyses are presented in tables 1 to 3, inclusive. Inspection shows that the total acidity increased regularly in the presence of sulfur. The greatest acidity was reached where sulfur alone was added. In this case, however, at the end of 32 weeks the value was only about 11 per cent of that found by Lipman, McLean and Lint (table 2) after 15 weeks of composting. Actual acidity (H^+ concentration, or pH) had practically reached its maximum after 12 weeks of composting. Conversion of the pH value to its equivalent of NaOH gives a value of 30 cc. or less 0.02 *N* alkali. Comparison of this value with that of total acidity shows that the latter was largely due to acid salts, rather than free sulfuric acid, as suggested by Lipman, McLean and Lint (8, p. 515). Accumulation of sulfates was much greater in the garden soil than in the silt loam, but it was as extensive where sulfur was supplied alone as where rock-phosphate was also added. Water-soluble P_2O_5 also increased to a much greater extent in the garden soil than in the loam, where both sulfur and rock-phosphate were added. These results probably depend upon the relative supplies of organic matter available for

TABLE 1
Changes in acidity of composts

TIME INTERVAL	SILT LOAM				GARDEN SOIL			
	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added
Actual acidity								
weeks	pH	pH	pH	pH	pH	pH	pH	pH
12		6.9	2.9	3.4	7.0	6.8	3.2	3.6
32	5.4	5.5	3.1	3.7	7.3	7.2	3.3	3.7
Total acidity 0.02 N alkaline								
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
0	14.6	14.6	14.6	14.6	17.1	17.1	17.1	17.1
4	15.8	8.5	24.8	44.9	17.1	10.7	70.5	53.4
8	11.5	9.4	106.8	115.3	21.4	8.6	128.2	102.5
12	35.0	20.0	343.9	282.8	15.8	11.5	208.0	249.9
32	21.4	21.4	730.5	551.1	11.5	13.7	527.6	376.0

TABLE 2
Changes in percentage of dilute HCl-soluble SO₃ of composts

TIME INTERVAL	SILT LOAM		GARDEN SOIL	
	Sulfur added	Rock-phosphate and sulfur added	Sulfur added	Rock-phosphate and sulfur added
weeks	per cent	per cent	per cent	per cent
0	Trace	Trace	Trace	Trace
4	0.25	0.42	1.17	1.00
8	0.42	0.58	2.00	1.83
12	0.42	0.67	2.25	2.42
32	1.10	1.25	2.75	2.75

TABLE 3
Changes in percentage of soluble P₂O₅ in composts

TIME INTERVAL	SILT LOAM				GARDEN SOIL			
	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added
Water-soluble P ₂ O ₅								
weeks	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
32	Trace	0.002	Trace	0.019	0.016	0.015	0.013	0.052
Citrate-soluble P ₂ O ₅								
0	0.06	0.34	0.06	0.34	0.09	0.32	0.09	0.32
4	0.06	0.34	0.07	0.33	0.10	0.34	0.11	0.35
8	0.06	0.30	0.05	0.29	0.12	0.36	0.11	0.33
12	0.04	0.30	0.05	0.35	0.11	0.34	0.10	0.32
32		0.27		0.38		0.28		0.42

bacteria in the composts. Citrate-soluble P_2O_5 remained unchanged, with the exception of an increase in the garden soil during the last period of the investigation. Had account been taken of the loss of dry matter by fermentation, this constituent must have shown a decrease on a percentage basis. It should be noted that in the similar work of Shedd (table 3) increase of citrate-soluble P_2O_5 in the complete compost was not detected until fermentation had proceeded for 7 months, and was first found to be extensive after 15 months, while in the tests of Lipman, McLean and Lint with greenhouse soil (8, table 2) the production of this constituent became very active between 3 and 4 months of fermentation. This difference may be due to differences of temperature and aeration.

A series of composts with manure only and with a mixture of manure and soil as basal materials also were prepared. Fresh horse manure was air-dried and mixed with the other compost materials. The composts were inoculated with a water extract of fresh manure and allowed to ferment for 15 weeks. Although the total acidity increased three to seven-fold where sulfur was added, the percentage of citrate-soluble P_2O_5 remained practically stationary. The duration of this experiment should be noted in comparison with that of experiments subsequently described.

A similar test of manure composts was conducted on a larger scale.³ Fresh cow and horse manures were freed from coarse litter and mixed in the proportion of 2 to 1, by weight. Samples were taken immediately for bacterial counts on agar media and for chemical analysis. Six portions of 4540 gm. (10 lbs.) each were then placed in glazed stoneware jars of a capacity of 2 gallons each. Additions were incorporated as follows:

- Jar 1. Nothing.
- Jar 2. Nothing (eventually received 32 gm. $Ca(H_2PO_4)$ and 146.7 gm. $CaSO_4 \cdot 2H_2O$).
- Jar 3. Nothing (eventually received 65 gm. rock-phosphate and 21.4 gm. sulfur).
- Jar 4. 150 gm. rock-phosphate and 50 gm. sulfur.
- Jar 5. 150 gm. rock-phosphate.
- Jar 6. 50 gm. sulfur.

The added materials had been sifted through a 100-mesh sieve. After covering with burlap bags, the contents of the jars were incubated at room temperature. At intervals of 32 and 87 days the composts were mixed and the control and those which had received additions were sampled and analyzed as at the beginning. It was impracticable to continue this experiment longer. Loss of organic matter by fermentation was determined by weighing the composts before and after sampling, thus rendering possible the computing of data from successive analyses to the common basis of original dry matter. Extracts were prepared by the use of water and ammonium-citrate solution successively, after the manner of Tottingham and Hoffmann (14), excepting

³ J. A. Wolfram should receive credit for performing the laboratory and greenhouse work of this experiment.

the determination of citrate-soluble P_2O_5 at the last analysis, for which Lipman's method was followed. At the last analysis also in addition to titrating the usual water extract for acidity, a separate sample was extracted with hot water, following Lipman's method.

The data derived from this experiment are assembled in table 4. As shown in column 2 the loss of dry matter was greatest in the untreated manure. Naturally, the introduction of rock-phosphate reduced the percentage loss of dry matter. We have not retained the necessary data for computing the loss of organic matter for comparison with the control. The two composts just referred to continued to ferment actively in the second period of the investi-

TABLE 4

Composition of composts after various periods of fermentation based upon the original dry matter

COMPOST MATERIALS	RESIDUAL DRY MATTER		BACTERIAL COUNT PER GM			TOTAL ACIDITY (0.1 N alk.)				WATER-SOLUBLE SO_3			WATER-SOLUBLE P_2O_5			CITRATE-SOLUBLE P_2O_5		
	32 days	87 days	Beginning	32 days	87 days	Beginning	32 days	87 days		Beginning	32 days	87 days	Beginning	32 days	87 days	Beginning	32 days	87 days*
	per cent	per cent	mgm.	mgm.	mgm.	cc.	cc.	cc.	cc.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Manure.	71.2	58.5	206	214	158	20.5	11.6	9.7	19.7*	0.21	0.09	0.03	1.05	0.65	0.57	0.35	0.48	0.20
Manure, rock-phosphate and sulfur.	88.4	85.2	206	326	316	25.2	23.6	45	0.49	0.21	0.47	0.54	1.23	1.12	1.31	0.46	0.46	0.83
Manure and rock-phosphate.	80.0	70.7	206	400	247	24.0	11.2	10.4	14.8	0.20	0.07	0.04	1.28	0.82	0.43	0.46	0.58	0.21
Manure and sulfur.	91.0	89.2	206	356	366	32.2	28.6	40.2	39.6	0.40	0.36	0.54	1.13	1.11	0.99	0.37	0.21	0.92

* Lipman's method.

gation, while the losses of dry matter where sulfur was added were small during this period. The least loss occurred where sulfur was added alone. Where rock-phosphate was added alone the bacterial counts indicate decided stimulation of these organisms in the earlier period of the investigation, but the count falls in the second period. It will be noted that a somewhat smaller increase of bacteria in the composts containing sulfur was maintained throughout the experiment. These relations suggest important modifications of biological factors, dependent upon the kind of inorganic materials introduced into composting practice.

As regards total acidity, this factor had increased decidedly over 87 days where sulfur was present, but the effect was not apparent after 32 days. This indicates a relatively late predominance of the sulfur-oxidizing bacteria. The results of our method of extraction probably agree with those of Lipman's method as well as should be expected. It should be noted that the increase of acidity in these composts is not sufficient to promise much increase of availability of rock-phosphate also added. Excepting a high initial value in the sulfur-treated compost, the changes of sulfate parallel those of acidity.

Water-soluble P_2O_5 decreased appreciably in the manure and the compost with rock-phosphate, as fermentation progressed. This is in agreement with results previously reported (14). In the composts where sulfur was added the amount of water-soluble P_2O_5 remained practically unchanged. Citrate-soluble P_2O_5 showed significant variations only at the last analysis. It should be noted that the data there obtained are less than those for the corresponding water-soluble P_2O_5 in all cases. Compared among themselves the data of Lipman's method showed increased availability of P_2O_5 where sulfur was added with rock-phosphate. However, the available P_2O_5 in this case was about the same as where sulfur was composted alone. Similar relations obtain in the water-soluble P_2O_5 at the last analysis. The variations of citrate-soluble P_2O_5 are parallel to those of acidity, at the close of the fermentation period.

Application of the composts just described to greenhouse cultures of oats upon Miami silt loam and Plainfield sandy loam has confirmed the general indications of availability of P_2O_5 derived from the chemical analyses. Portions of each type of soil in the air-dried state, weighing 12.75 kgm. in the case of silt loam and 16.75 kgm. with sandy loam, were placed in boxes of galvanized iron 30 cm. square and 20 cm. deep. The inner surface of the boxes had been previously coated with paraffin. At this time, as previously indicated, additions of sulfur and rock-phosphate were incorporated with the residual manure of jar 3, and jar 2 received mono-calcium phosphate and calcium sulfate. These additions conveyed approximately the same proportions of P_2O_5 and sulfur as were involved in the other composts. Computed amounts of manure and composts, each equal to 100 gm. of the original fresh manure, were now tilled into the surface soil of the various boxes. A pedigreed strain of oats was then sown and the soils were sparingly and uniformly watered until the seedlings were 7 to 8 cm. high. The cultures were now reduced to 16 plants per box, uniformly distributed, and the water supply was controlled by weighing. At first the water plane was maintained at 30 per cent of the full water-holding capacity of the two soils. Two weeks later it was raised to 40 per cent and when signs of maturation appeared on the plants it was reduced. The cultures were rotated in periods of 2 or 3 days as to position upon the benches, for the purpose of counteracting effects of variations of temperature and illumination within the greenhouse. Plates 1 and 2 show the appearance of one set of the duplicate series of cultures conducted with each soil. These were taken after 15 weeks of growth and 2 weeks before harvesting. The plants

were crowded together as shown only for the purpose of photographing. When harvested the crops were dried at about 55°C. and exposed to the air at room temperatures for several days before weighing. Data of the yields

TABLE 5
Effects of composts on the yield of air-dried seed by oats

MANURIAL ADDITIONS	NOTHING	FER- MENTED MANURE	FER- MENTED MANURE, CaSO ₄ AND Ca (H ₂ PO ₄) ₂	FER- MENTED MANURE, SULFUR AND ROCK- PHOSPHATE	COMPOST OF MANURE, SULFUR AND ROCK- PHOSPHATE	COMPOST OF MANURE AND ROCK- PHOSPHATE	COMPOST OF MANURE AND SULFUR
On silt loam							
Culture 1 (gm.)....	7.4	9.3	11.7	11.2	12.3	9.4	10.0
Culture 2 (gm.)....	8.3	9.2	9.7	11.1	14.0	10.0	8.0
Average (gm.)....	7.8	9.3	10.7	11.2	13.2	9.7	9.0
Relative (per cent).		100	115	120	142	104	97
On sandy loam							
Culture 1 (gm.)....	5.8	7.3	5.3	8.2	9.3	6.6	11.5
Culture 2 (gm.)....	5.3	5.2	5.5	8.8	10.1	7.9	10.3
Average (gm.)....	5.6	6.3	5.4	8.5	9.7	7.3	10.9
Relative (per cent.)..		100	86	134	154	116	173

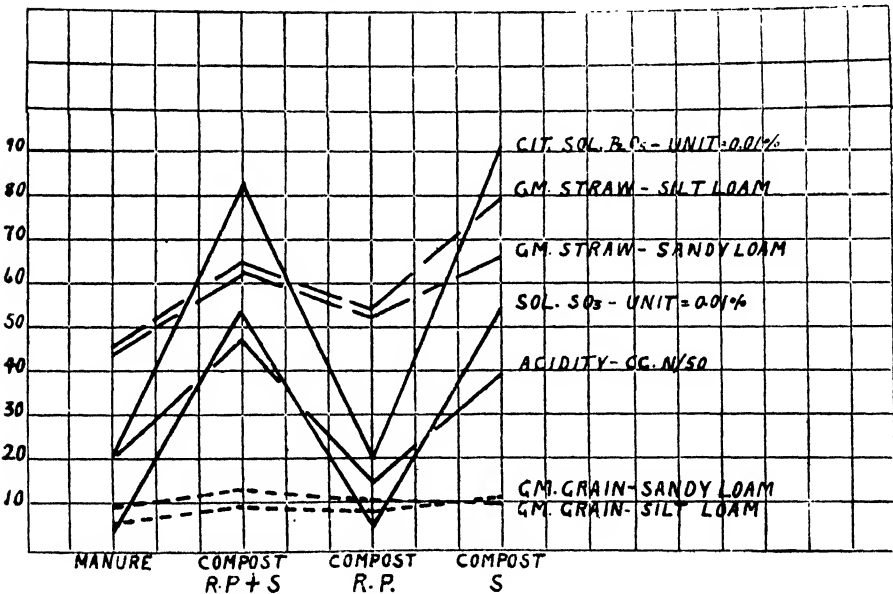


FIG. 1. COMPARISON OF YIELD (SOIL CULTURES) WITH COMPOSITION OF 12-WEEK COMPOSTS

are assembled in table 5. Inasmuch as the predominant effects of variations in the supply of P_2O_5 appear in seed production, we have omitted yields of straw from the table. Figure 1 gives a comparison of yields of both grain and straw with acidity, water-soluble SO_3 and citrate-soluble P_2O_5 of the composts.

The general agreement between high yields of grain and straw and increase of citrate-soluble P_2O_5 , water-soluble SO_3 and acidity in the composts is readily apparent. With the exception of seed production on the silt loam, it appears that sulfur alone was as effective in promoting crop yields as its combination with rock-phosphate. Addition of rock-phosphate and sulfur to the manure at the time of applying the latter led to better yields than those obtained from the rock-phosphate compost. It will be observed that the use of calcium sulfate and mono-calcium phosphate was attended by relatively poor yields. It seems probable that this was due to excessive development of soil acidity from the latter salt.

Another composting experiment with manure was arranged on the same scale of magnitude as that just reported. Considering the relatively low development of acidity from sulfur in the previous test as compared with the results of Lipman and his coworkers, this test was conducted over a longer period than the preceding one. Furthermore, an additional compost of rock-phosphate and sulfur was conducted with weekly mixing, to promote aeration. As before, rock-phosphate and sulfur were used in the proportion of 3 to 1, as early used by Lipman (8, p. 513), and the proportions between these and the dry matter of the manure were equal to Lipman's proportions between these and the soils used. The sulfur was passed through an 80-mesh and the rock-phosphate through a 60-mesh sieve. A moisture content of 76.4 per cent was found in the mixture of fresh horse and cow manures. Portions of compost materials were prepared as follows:

- Jar 1. 4540 gm. manure.
- Jar 2. 4540 gm. manure (receiving ultimately 300 gm. acid-phosphate).
- Jar 3. 4540 gm. manure (receiving ultimately 150 gm. rock-phosphate and 50 gm. sulfur).
- Jar 4. 4540 gm. manure and 150 gm. rock-phosphate.
- Jar 5. 4540 gm. manure and 50 gm. sulfur.
- Jar 6. 4540 gm. manure and 150 gm. rock-phosphate and 50 gm. sulfur.
- Jar 7. 4540 gm. manure and 150 gm. rock-phosphate and 50 gm. sulfur, to be mixed weekly for aeration.

After standing at room temperature for 97 days, and again after 129 days, the manures and composts were mixed and sampled for moisture determinations. On the latter date they were also subjected to the usual chemical analysis, the delayed additions now having been made. Lipman's analytical methods were used (8, p. 511). Fifty grams of the sample were extracted for determining citrate-soluble P_2O_5 in the material from jars 1 and 2, but 100 gm. were used in all other cases. The data obtained are presented in table 6.

Inspection of the data shows that the percentage of total P_2O_5 which became soluble in ammonium-citrate solution where rock-phosphate was composted was decidedly increased by the supplementary application of sulfur. While the acidity was reduced in the latter compost by frequent mixing, the solubility of the P_2O_5 was decidedly enhanced thereby. If multiplied by 5, to bring them

to approximate equivalence with Lipman's data (basis of 120 gm. dry matter), the maximum acidities of the sulfur-treated composts are about equal to that found by Lipman, McLean and Lint in their compost of garden soil with rock-phosphate and sulfur after a like period of 18 weeks (1, table 2), but they found twice as great acidity where sulfur was composted alone. The use of sulfur alone did not promote solubility of the P_2O_5 as it did in the preceding experiment. These results agree with the observation of Lipman and his associates (8, p. 518) that sulfonation gains intensity after about 18 weeks.

The availability of P_2O_5 in these composts was tested with sand cultures of barley, following the method of Lipman and McLean (7). Sand of unusual purity supplied by the Ottawa Silica Company of Illinois was used, after thorough washing with water. It consisted of spherical particles screening be-

TABLE 6

Composition of fermented manure and composts of manure with sulfur and phosphates

COMPOST MATERIALS	DRY MATTER		LOSS OF ORGANIC MATTER	ACIDITY ON BASIS OF 100 GM. OF ORIGINAL MANURE	CITRATE- SOLUBLE P_2O_5	TOTAL P_2O_5	SOLUBILITY OF P_2O_5
	96 days	129 days					
	per cent	per cent	per cent	cc 0.02 N alk.	per cent	per cent	per cent
Fermented manure.....	31.1	44.3	53.7	28	1.50	2.72	55.2
Fermented manure and acid- phosphate	34.2	45.2	49.4	488	5.34	6.61	80.8
Fermented manure, rock- phosphate and sulfur ...	24.9	31.4	48.9	27	1.19	7.48	15.9
Compost of manure and rock-phosphate.....	28.3	33.0	52.0	24	1.26	8.05	15.7
Compost of manure and sulfur.	31.2	37.6	23.7	426	0.89	1.65	53.9
Compost of manure, rock- phosphate and sulfur...	37.3	45.6	18.6	464	1.24	5.14	24.3
Compost of manure, rock- phosphate and sulfur, mixed weekly.....	47.3	61.5	18.8	259	1.48	4.87	30.4

tween 60 and 70-mesh, and had a water-holding capacity of 23.4 per cent. Portions of 3.5 kgm. were placed in glazed stoneware jars of 0.5-gallon capacity. The following salt mixture was then incorporated with each portion of sand:

3.9 gm. $CaCO_3$
 0.8 gm. K_2SO_4
 0.4 gm. $MgSO_4 \cdot 7H_2O$
 0.1 gm. $Fe_2(SO_4)_3$

$NaNO_3$ was applied later to the extent of 1.2 gm. per jar, 0.4 gm. being added in solution soon after the seedlings appeared and the rest 11 days later. Manurial additions of the composts equivalent to 1 gm. of rock-phosphate in

composts containing the latter were tilled into the surface sand of the culture jars. A pedigreed strain of barley was sown and distilled water was applied to the extent of 36 per cent of the full holding capacity of the sand. After the seedlings were well established the cultures were reduced to 6 plants per jar and the water plane was increased to 60 per cent of saturation. The crops were photographed (plate 3) after 82 days of growth and were harvested one week later. Data of the manurial treatments and yields of dry matter appear in table 7. The results also appear graphically in figure 2.

It will be noted that direct use of rock-phosphate and sulfur with the fermented manure produced as good yields of seed as the usual composting treatment of these materials. There was an increased yield from the aerated complete compost, but this increase was not proportionate with the increase of

TABLE 7

Yields of barley from fertilized sand cultures supplemented by composts

JAR NUMBER	MANURIAL ADDITION	SEED			STRAW	
		gm.	gm.	gm.	gm.	gm.
1	Fermented manure.....	9.10	3.2		10.4	
2	Fermented manure.....	9.10	2.7		10.2	
3	Fermented manure 9.73 gm., and acid-phosphate....	2.00	5.9		12.0	
4	Fermented manure 9.73 gm., and acid-phosphate....	2.00	6.0		12.7	
5	Fermented manure 14 gm., rock-phosphate 1.0 gm., and sulfur.....	0.33	5.8		11.6	
6	Fermented manure 14 gm., rock-phosphate 1.0 gm., and sulfur.....	0.33	5.6		12.3	
7	Compost of manure and rock-phosphate.....	16.20	3.3		9.9	
8	Compost of manure and rock-phosphate.....	16.20	1.6		8.5	
9	Compost of manure and sulfur.....	17.33	5.9		13.4	
10	Compost of manure and sulfur.....	17.33	5.8		12.5	
11	Compost of manure, rock-phosphate and sulfur.....	17.57	5.5		13.1	
12	Compost of manure, rock-phosphate and sulfur.....	17.57	5.8		12.2	
13	As above, but aerated by weekly mixing.....	13.13	6.8		13.2	
14	As above, but aerated by weekly mixing.....	13.13	5.9		12.6	

citrate-soluble P_2O_5 . We believe these results indicate that available P_2O_5 produced by composting is subjected to competitive biological and other influences in the soil, which may alter its condition before absorption by the plant occurs. The results are very favorable for the complete composts, in so far as they produced yields equal to those derived from the use of acid-phosphate. As in the case of sandy loam of the previous culture experiment, sulfur alone, as a composting addition, has shown remarkable efficiency in grain production. It was as effective as any of the other manurial treatments, unless exception be made of the questionable superiority of the aerated compost. This predominant effect of sulfur raises the question as to whether it functions under such conditions by liberating available P_2O_5 from the manure, supplementing the efficiency of small supplies of the latter, or by acting directly as a nutrient,

after oxidation. If the first of these conditions obtains it appears, in view of the results of analysis, that it may have been brought about after the compost was applied to the barley cultures in this experiment. Exact comparison of the P_2O_5 absorbed by the cultures which received sulfur compost with that absorbed where manure alone was applied would be of interest, but material was not reserved for these determinations. Computation shows the application of citrate-soluble P_2O_5 per culture for the vertical order of manurial additions of table 7 to have been as follows: 60 mgm., 342 mgm., 68 mgm., 68 mgm., 58 mgm., 99 mgm., and 119 mgm. Assuming an average of 0.8 per cent P_2O_5 in barley seed, the absorption of this constituent by this part of the plants would have been only 24 mgm. in the average manured culture and 46 mgm. in the one which received sulfur compost. According to Pember (9) the minimum P_2O_5 requirement of 6 barley plants, as determined by the water-culture method, is 45 mgm. These data indicate that all the composts

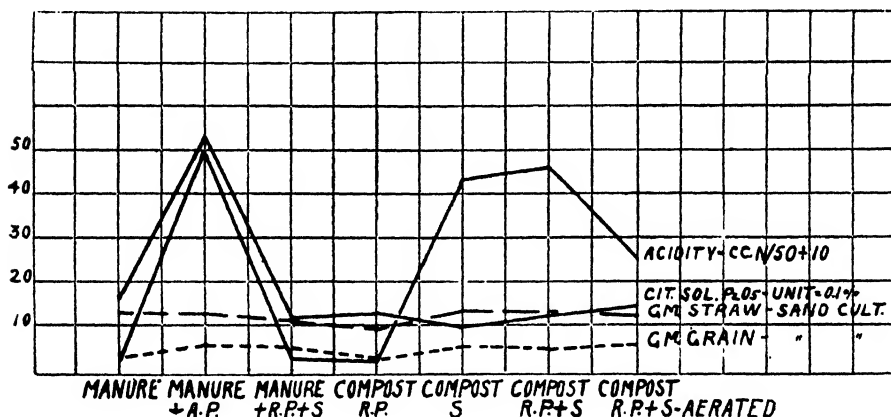


FIG. 2. COMPARISON OF YIELD (SAND CULTURES) WITH COMPOSITION OF 18-WEEK COMPOSTS

as applied conveyed liberal supplies of available P_2O_5 . We are inclined to believe, however, that the manure applied alone and the compost containing only rock-phosphate suffered reversion of this form of P_2O_5 in the presence of $CaCO_3$ in the culture jars. The same effect is also likely to arise, of course, from the natural alkaline fermentation of manure. Hence, it appears quite possible that the beneficial nutrient effects of sulfur composts already pointed out are due to the production and maintenance of an adequate supply of available P_2O_5 from the manure used, as a result of acidity arising from sulfification.

After samples of these composts which had been dried at $98^\circ C$. had been kept about one year they were analyzed for total sulfur, sulfate sulfur, sulfite and thiosulfate sulfur combined and elemental sulfur (CS_2 extraction). The analysis included the control manure, the compost with sulfur and the un-aerated compost with sulfur and rock-phosphate. Less than 0.1 per cent of sulfite and thiosulfate sulfur occurred in any case. Of the total sulfur

of the manure only a trace was present as SO_3 , while in the sulfur compost the latter formed 36.9 and in the complete compost 45.6 per cent of the total sulfur. The corresponding values for elemental sulfur were 0.0, 50.6 and 42.8 per cent of the total sulfur, respectively.

In field trials we have tested the efficiency of composts as compared with the direct application of rock-phosphate and sulfur with manure. The results are inconclusive, due, apparently, to the considerable reserve of P_2O_5 in Miami silt loam, to which soil the trials have been thus far confined. In the season of 1920, barley on plots 1 by 2 rods in dimensions gave slightly better yields of grain where the manurial treatment consisted of fermented manure, rock phosphate and sulfur than where the corresponding compost was applied. The applications were equivalent to 8 tons of fresh manure per acre, reinforced with 40 pounds of rock-phosphate and 13.7 pounds of sulfur per ton.

TABLE 8

Yields of air-dry matter from greenhouse crops to which sulfur was added with or without lime

FERTILIZER ADDITION	ON MIAMI SILT LOAM					ON PLAINFIELD SANDY LOAM
	Clover	Mustard		Rape	Turnip	Clover
		Seed	Straw			
	gm.	gm.	gm.	gm.	gm.	gm.
Nothing.....	55.0	2.7	44.0	19.3	13.9	61
10 gm. CaCO_3	54.1	2.8	49.0	13.0	11.8	102
0.5 gm. sulfur.....	70.5	4.0	42.5	21.8	16.2	54
0.5 gm. sulfur and 10 gm. CaCO_3	59.7	1.6	36.6	13.3	10.9	96
1.0 gm. sulfur.....	69.2	2.4	36.0	20.8	16.5	59
1.0 gm. sulfur and 10 gm. CaCO_3	60.2	3.2	32.0	11.6	15.5	102
3.0 gm. sulfur.....	69.2	4.5	46.5	22.4	14.7	52
3.0 gm. sulfur and 10 gm. CaCO_3	70.1	2.5	54.0	14.9	13.1	106
5.0 gm. sulfur	70.9	5.3	58.0	20.9	12.1	41
5.0 gm. sulfur and 10 gm. CaCO_3	63.5	1.8	44.2	14.1	10.5	96

Shedd (11) obtained beneficial effects on the yield by applying elemental sulfur to various crops in comparison with sulfates. Reimer and Tartar (9) have conducted similar comparisons with alfalfa in southern Oregon, with remarkable results from sulfur on the basic soils used. They consider an application of 40 pounds of sulfur yearly to be sufficient.

We have used elemental sulfur as a fertilizer in both greenhouse and field tests. In the former case plants were selected from species which absorb sulfur freely. Sulfur was applied in amounts ranging from 0.5 to 5 gm., either with or without 10 gm. of CaCO_3 , per portion of soil. The latter consisted of either 15 kgm. of Miami silt loam or 20 kgm. of Plainfield sandy loam in cypress boxes. These additions are equivalent to 1 ton of limestone and 100 to 1000 pounds of sulfur per acre. The data of sulfur additions and yields are assembled in table 8.

Apparently the sandy loam was deficient in lime. The benefits from sulfur were generally considerable, but the rate of 100 pounds per acre was as efficient as the larger applications.

The use of sulfur on field plots of barley has given results which confirm the beneficial effect of the element, apart from application with rock-phosphate. Applications of the fertilizer materials were tilled into plots of 2 square rods area (1 by 2 rods) each on sandy silt loam in 1919. No farm manure had been applied to this land for four years. Barley was sown in 1919 and 1920, with no fertilizer applications the latter year. Table 9 contains the data of treatment and yields. The striking effect of lime in promoting maturation of the crop in 1919 is shown in plate 4. Heading out was completed several days earlier on the limed than on the unlimed plots.

TABLE 9

Yields of barley grain from plots treated with sulfur, with and without lime

PLOT NUMBER	FERTILIZER ADDITIONS OF 1919, PER ACRE	YIELD PER ACRE*	
		1919	1920
		<i>bu.</i>	<i>bu.</i>
27	Nothing	41.7	36.7
13	1000 lbs. marl.....	45.0	40.9
26	500 lbs. rock-phosphate.	48.3	43.4
12	1000 lbs. marl and 500 lbs. rock-phosphate	47.5	38.4
23	500 lbs. rock-phosphate and 50 lbs. sulfur	43.4	35.0
11	1000 lbs. marl, 500 lbs. rock-phosphate and 50 lbs. sulfur....	46.7	35.0
17	100 lbs. sulfur.....	54.2	50.0
16	1000 lbs. marl and 100 lbs. sulfur	55.0	50.8
24	500 lbs. rock-phosphate and 100 lbs. sulfur.....	45.8	35.0
14	1000 lbs. marl, 500 lbs. rock-phosphate and 100 lbs. sulfur. ...	51.7	42.5
25	500 lbs. rock-phosphate and 300 lbs. sulfur....	51.7	38.4
15	1000 lbs. marl, 500 lbs. rock-phosphate and 300 lbs. sulfur....	47.5	43.4

* 48 lb. per bushel.

The beneficial effect of liming noted in the time of maturation is reflected also in the yields of barley. As regards the use of sulfur with rock-phosphate, 100 pounds per acre gave greater yields than either 50 or 300 pounds. However, in harmony with the results from sand cultures already presented, sulfur at the medium rate of application was as efficient alone as when supplemented with either rock-phosphate or rock-phosphate and lime.

As a practical consideration, the use of sulfur was profitable in this test. With the then current price of 8 cents per pound for sulfur, it appears from table 9 that a 100-pound application of the element produced a 26-bushel increase of barley over the yield from untreated soil in the two successive years, at a cost of 31 cents per bushel.

Although this effect of sulfur seems to indicate an important function of the element in rendering P_2O_5 available from latent forms in soils and composts,

the possibility must not be overlooked that it may function also in the form of oxidation products. These may be either stimulating intermediate forms or the more strictly nutrient sulfates ultimately formed. Reimer and Tartar (10, p. 35) emphasize the function as sulfates. The results of our determinations of forms of sulfur in the composts applied to sand cultures indicated little importance quantitatively for the intermediate products of oxidation.

In other field experiments on Miami silt loam, now in progress where steamed bone meal is used in conjunction with potassium salts and gypsum, no increase in yield of barley, either straw or grain, has been observed over a period of 7 years. These results are introduced here as evidence of probable lack of effect from sulfur as an oxidation product in the form of sulfates, in the sulfur test just reported. It should be remembered, nevertheless, that the sulfate requirement of cereals is low as compared with that of alfalfa, the crop used in the work of Reimer and Tartar.

As a further test of the importance of sulfates in the action of elemental sulfur, we have used the two in comparison, and also superimposed sulfur upon sulfates, in a greenhouse experiment.⁴ Portions of 15 kgm. of Miami silt loam were placed in the galvanized iron boxes previously described. After incorporating the desired additions, with the exception of NaNO_3 , the soils were sown to oats and watered to 20 per cent of their holding capacity. The seedlings were reduced to uniform numbers per culture, and the water plane was gradually raised to 60 per cent of saturation. It was intended to reduce gradually the water plane as maturation set in, but early in the period of seed formation the cultures became quite dry for a brief period. This condition, and the unusually low light intensity, even for the season of growth (October 16 to March 1), may account for the erratic yields of seed obtained from duplicate cultures. The usual care was taken to rotate the cultures as to position within the house. Growth was rapid and apparently normal, excepting a form of leaf burn where sulfur was superimposed upon sulfates, and uniformly slow filling of the seed.

The complete fertilizer consisted of 5 gm. NaNO_3 (applied in portions to the growing plants) 5.85 gm. KCl and 10 gm. $\text{Ca}_3(\text{PO}_4)_2$ culture. Lime was added in the form of 10 gm. CaCO_3 per culture. Sulfur, sodium sulfate and calcium sulfate were applied in equivalent amounts upon three planes, designated as "low," "medium" and "high." These applications were equivalent to 0.5, 1.4 and 4.2 gm. sulfur per culture or 33, 100 and 300 pounds per acre, respectively.

Inasmuch as the yields of seed were quite variable from cultures treated in duplicate, we shall neither present the data in full nor comment extensively upon them. The yields of straw gave consistent duplicate values, with the exception of the supplementary treatment of sulfur added to sulfates. For

⁴ This experiment was performed by S. Lepkovsky, as also the determination of forms of sulfur in the composts applied to sand cultures.

the most part, the total seed yields from various treatments varied in much the same direction and order as the corresponding yields of straw.

As to general indications from this test, gypsum was superior to sulfur, whether or not lime was added. It was also superior to sulfate of sodium on the limed soil. This is in agreement with previous observations (13, p. 248). Liming depressed the efficiency of gypsum; moreover, all of the erratic seed yields occurred where lime was applied. It thus appears that an excess of lime on the soil may not only depress the availability of P_2O_5 , but also interfere with the functions of sulfate of lime. Such interference as the latter might ensue if, as suggested by one of the writers (5, p. 437), gypsum functions in an important manner by combining the supply of sulfur with calcium in molecular form. Thus, an excess of Ca as $Ca(HCO_3)_2$ in the soil solution might depress the absorption of $CaSO_4$, and hence of sulfur, by the plant.

The best results from elemental sulfur attended the medium application of 100 pounds per acre. This rate, in view of agreement with previous tests, therefore seems to be fairly well established as optimal for Miami silt loam under our experimental conditions. Inasmuch as sulfur was ineffective when superimposed upon sulfates in this test, it might seem that the results lend support to the belief that the element functions largely through conversion to sulfates. It is quite possible, however, that the presence of sulfates interfered with the oxidation of sulfur or otherwise prevented its favorable effect upon the availability of P_2O_5 .

It follows logically that the use of sulfur in field practice will sooner or later tend to such depletion of P_2O_5 as to necessitate the supplementary use of phosphate fertilizer. The same will be true of lime, and probably other soil constituents, to a greater or less degree.

SUMMARY

Soil composts with sulfur added developed much acidity in 32 weeks. The values were greater for sulfur alone than where rock-phosphate also was added. Dissociated acid formed but a small portion of the total acid.

Composts of sulfur with horse manure showed appreciable increase in acidity, but no increase of citrate-soluble P_2O_5 , after 15 weeks.

Sulfur in composts with 4.54 kgm. of manure decreased the loss of organic matter by fermentation. Increased bacterial counts in these composts were maintained to the conclusion of 12.5 weeks. Acidity doubled in the rock-phosphate and sulfur compost over this period, but was unchanged after 4.5 weeks. The variations of water-soluble SO_3 were in the same direction as those of acidity, increasing where sulfur was added and decreasing in the other cases. Citrate-soluble P_2O_5 approximately doubled where sulfur was added, the percentage being based upon the common basis of original dry matter of the composts. In the other cases the percentage of this constituent decreased approximately one-half. Yields of oats from soil cultures to which these com-

posts were applied agreed generally with the results of analysis of the latter, producing the greatest yields where sulfur had been applied. On Plainfield sandy loam the yield of seed where sulfur compost was applied was as great as where the corresponding treatment included rock-phosphate. Increased yields of oats were obtained also from the application of rock-phosphate and sulfur with fermented manure.

Similar composts which fermented 18.5 weeks showed 60 per cent greater availability of P_2O_5 (solubility in ammonium-citrate solution), by the addition of sulfur to rock-phosphate. When aerated by weekly mixing the corresponding increase of available P_2O_5 was 90 per cent, although the total acidity was decreased thereby. The total acidity developed in the complete compost was nearly as great as that resulting from an equivalent addition of acid-phosphate to the fermented manure. In this experiment, the use of sulfur alone did not increase the proportion of citrate-soluble P_2O_5 in the manure. Application of these fermented manures and composts to sand cultures of barley led to equal yields from the rock-phosphate and sulfur compost and a corresponding portion of fermented manure supplemented by acid-phosphate. These yields were not superior to those from sulfur compost and manure reinforced by rock-phosphate and sulfur.

The process of sulfification is inactive after 12 weeks, but becomes very active after 18 weeks.

In greenhouse trials on Miami silt loam and Plainfield sandy loam with clover and cruciferae, sulfur increased growth on the former soil. One hundred pounds of sulfur per acre was as effective as more.

Barley on field plots of sandy silt loam apparently in need of lime produced increased yields of seed by the application of sulfur. Sulfur alone was as effective as its combinations with marl and rock-phosphate. One hundred pounds of sulfur per acre was as effective as 50 or 300 pounds.

Sulfate of lime produced better yields of oats upon Miami silt loam than equivalent amounts of sulfate of soda or sulfur, when these supplemented the usual complete fertilizer in greenhouse cultures. Liming depressed the efficiency of sulfate of lime under these conditions. One hundred pounds of elemental sulfur was more effective than either one-third or three times as much. Benefits from elemental sulfur were not apparent when it was superimposed upon the sulfates for application.

It appears probable that sulfur functions as a fertilizer both by oxidation to the nutrient SO_3 and by producing, through oxidation, an acid condition favorable to the production of available P_2O_5 . These processes occur in composts of sulfur and rock-phosphate. They also, doubtless, continue when the compost materials are tilled into the soil.

It remains to be proved whether the efficiency of sulfur is any greater when it is composted with rock-phosphate and manure than when these materials are added simultaneously to the soil.

Adequate consideration of the use of sulfur as a fertilizer must recognize its tendency to deplete the stock of CaO , P_2O_5 and other soil constituents.

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PLATE 1
OATS ON MIAMI SILT LOAM
In each case, culture 1 of table 5

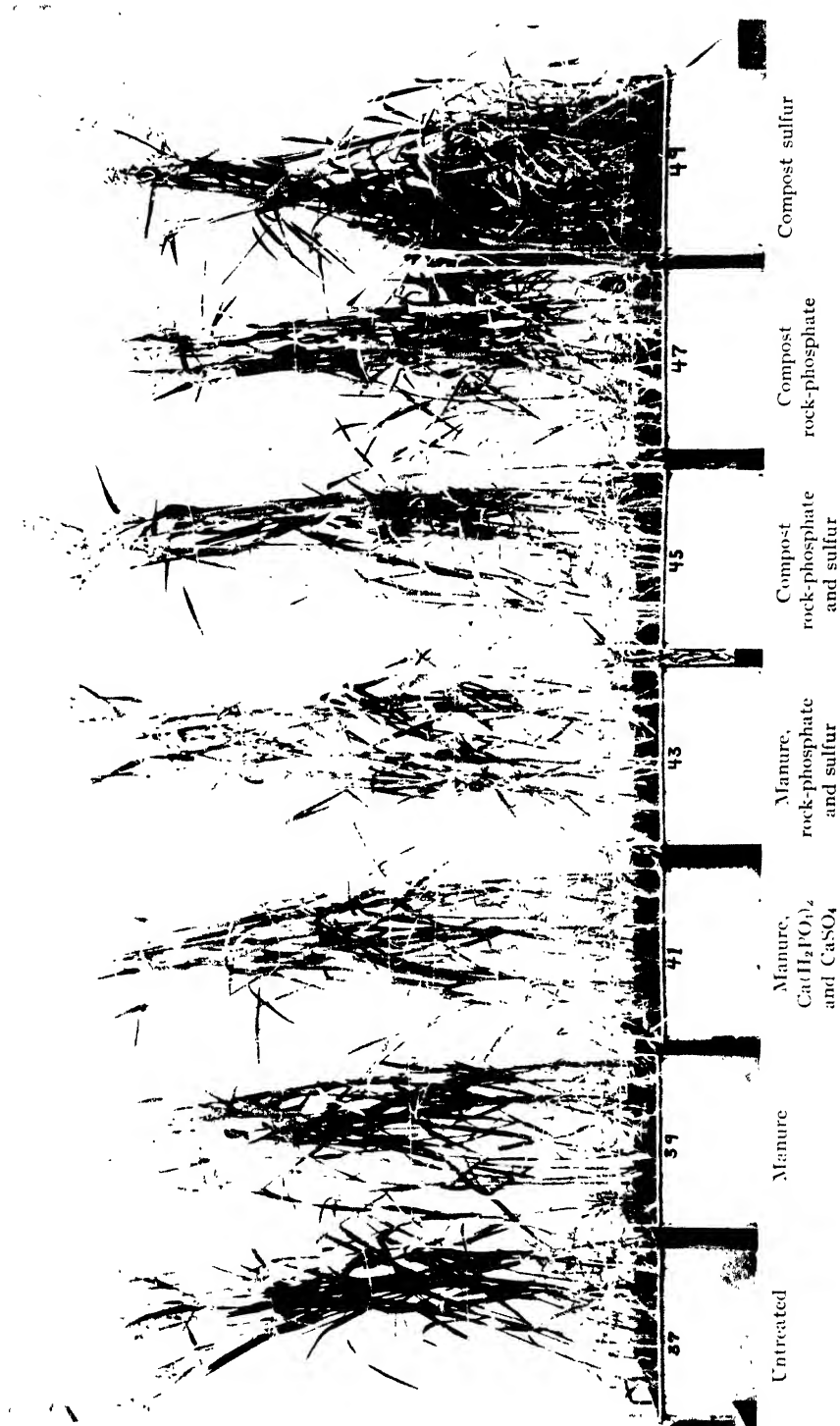


PLATE 2
OATS ON PLAINFIELD SANDY LOAM
In each case, culture 1 of table 5

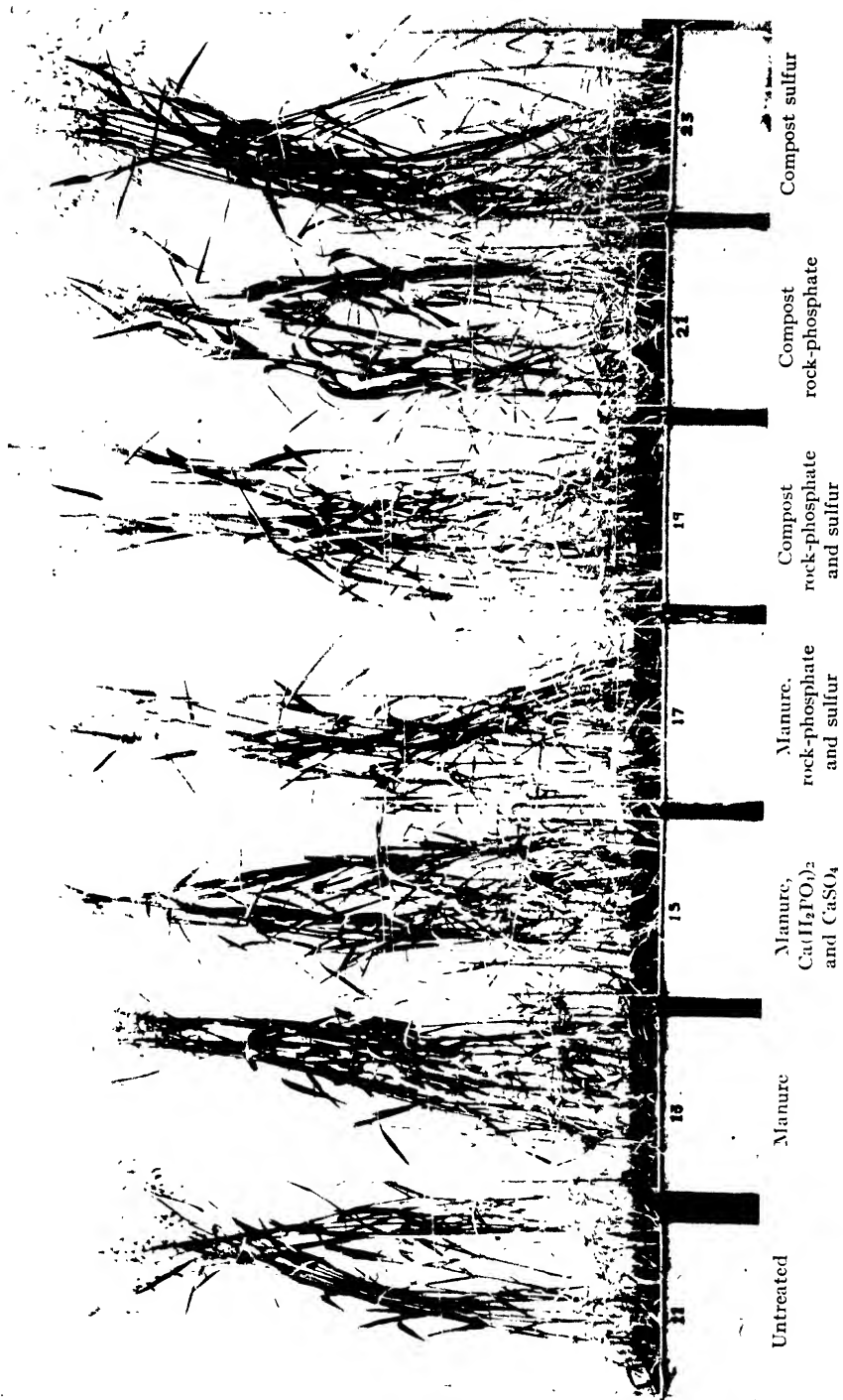


PLATE 3

BARLEY ON SAND WITH ADDITION OF A PHOSPHORUS-FREE SALT MIXTURE

Odd-numbered jars of table 7

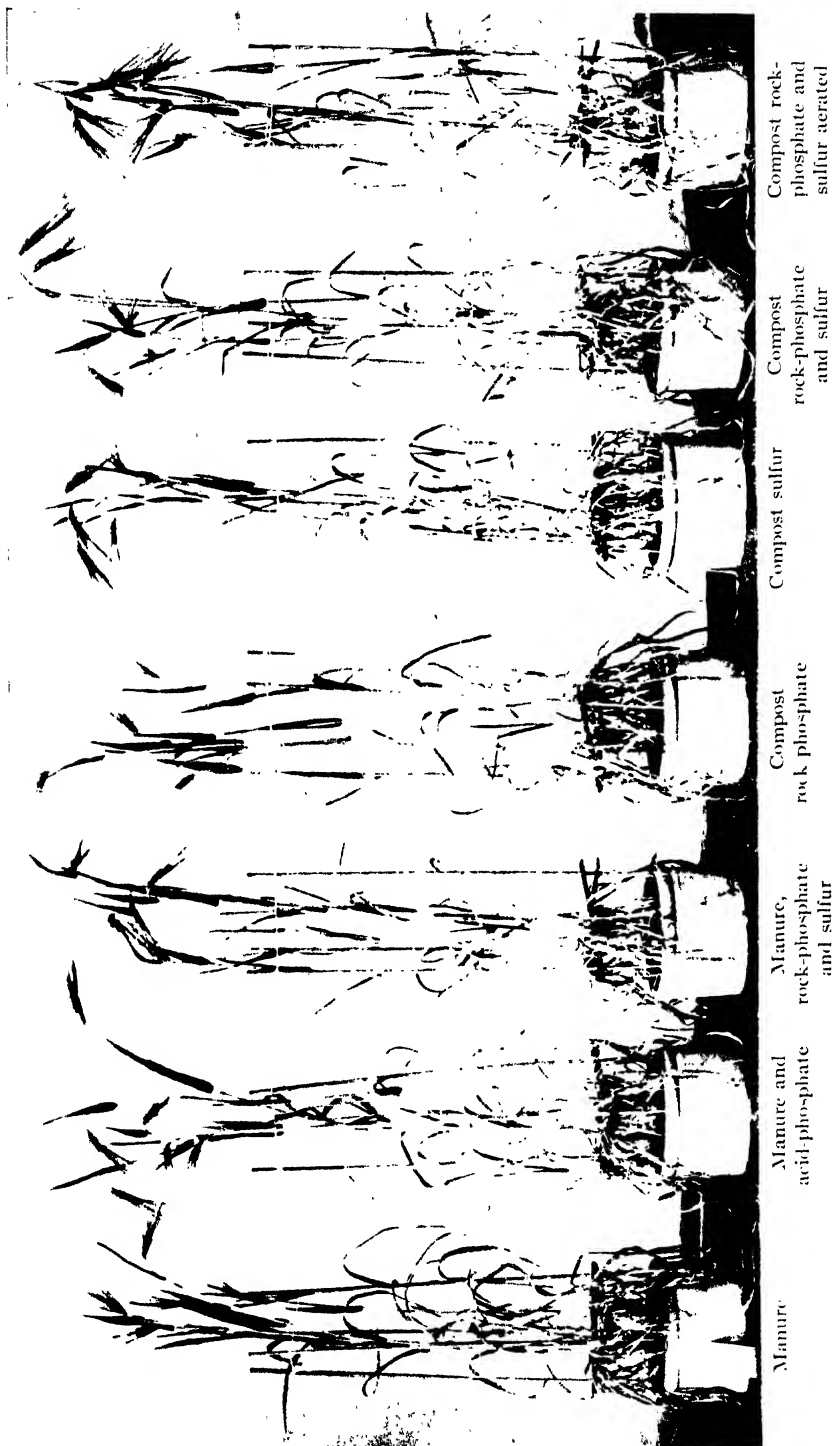


PLATE 4

MATURATION DIFFERENCE IN BARLEY OF SULFUR TRIALS, 1919

Left tier, limed; right tier, unlimed



A COMPARISON OF INOCULATED AND UNINOCULATED SULFUR FOR THE CONTROL OF POTATO SCAB¹

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INTRODUCTION

It has long been known that the presence or absence of the potato scab organism (*Actinomyces chromogenus* Gasperini) is determined largely by the soil reaction. As a result of this knowledge studies on control measures have been based to a large extent on determining some practical method whereby the soil reaction can be so changed as to produce conditions unfavorable for the development of scab. Various substances have been tested in this connection and of these sulfur has been found to be the most promising. In its use, however, much contradictory evidence has resulted. In some instances very good control was obtained while in others it proved practically worthless. In a previous paper (8) the statement was made that some of the reported failures of sulfur to control scab may have resulted from the fact that the sulfur was not oxidized. It is recognized that environmental conditions play an important rôle in the oxidation of sulfur but the presence of sulfofying micro-organisms would appear to be of even greater importance. Boullanger and Dugardin (1) found that the effect of sulfur on crop yields was not as marked on sterilized as it was on unsterilized soils. They conclude that this difference resulted from the fact that the oxidation of the sulfur was brought about by bacterial activities. As a result of adding sulfur to sterilized and unsterilized soil Demolon (4) found very little sulfur to have been oxidized in the sterilized soil while a considerable amount was oxidized in that which was unsterilized. Brown and Kellogg (2) found that each soil has a definite sulfofying power. They also found that while the formation of a small amount of sulfates was brought about by chemical action, the presence of bacteria is essential. Lipman and his co-workers (6) have shown that elemental sulfur is readily oxidized in soils containing sulfofying bacteria. In a later paper (7) as a result of a comparison of untreated soils with soils that had been sterilized and inoculated, and others unsterilized and uninoculated, they demonstrated the biological factor to be influential in the oxidation of sulfur.

From this brief summary of a portion of the literature on the oxidation of sulfur in soils the importance of the presence of sulfofying organisms is appar-

¹ Paper No. 12, of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

ent. It is very probable that the lack of these organisms in the soil might determine to a large extent the success or failure of sulfur to control scab. The experiments herein reported were conducted to determine to what extent this might be true.

EXPERIMENTAL

In the experiments here reported each treated plot was adjoined by an untreated check plot and the size of the plots was made large enough to reduce as far as possible the error arising from soil variations. Except for the sulfur applications all plots in an experiment were treated alike as regards fertilization and cultivation.

Inoculated and uninoculated commercial flour sulfur was used. Inoculation was effected by means of a thorough mixing of the commercial flour with 1 per cent of soil from a compost heap known to be well supplied with the sulfur-oxidizing organisms. The sulfur applications were made with a grain drill just after harrowing and just before planting. This method proved very satisfactory, as it not only insured uniform distribution but at the same time worked the sulfur thoroughly into the upper several inches of soil.

The experiments were conducted with the Irish Cobbler, a variety known to scab very severely. When harvested, the crop was divided into three classes, namely, clean, salable and unsalable scabby. The first class was made up of all tubers free from scab. The tubers in the second class showed only a moderate infection while those in the last class were for most part covered with scab lesions.

Soil samples were taken before the sulfur applications were made and again at digging time. In taking the soil samples borings were made to a depth of $6\frac{1}{2}$ inches at intervals of approximately 15 feet. The hydrogen-ion concentration of water extracts of the soil samples was determined colorimetrically following the method devised by Gillespie (5). The indicators used were those recommended by Clark and Lubs (3). In preparing the water extracts of the soil samples to be tested, the method followed was essentially the same as described in the previous paper (8).

The tables and diagrams show the yield per acre as well as the relation of clean, salable and unsalable scabby tubers to the hydrogen-ion exponent. The data given are averages obtained from at least three replications of each treatment and from four check plots.

Experiment I

Section A. The soil on which this experiment was conducted is a Sassafras loam. In 1915 an application of ground limestone was made at the rate of 3 tons per acre. Following this treatment the field was in grass in 1916 and 1917, and in potatoes in 1918 and 1919. In the latter year there was a uniform infection of scab over the entire field, a greater part of the crop being unsalable.

The field was divided into two parts that may here be designated as sections A and B. On the former a comparison was made of 600-pound applications of inoculated and uninoculated sulfur. On section B the amount employed was reduced to 300 pounds. These two sections will be discussed in turn.

The effect of the 600-pound application on the total yield and the number of clean and scabby potatoes is shown in table 1. It will be observed that the plots treated with both the inoculated and the uninoculated sulfur showed increases in yield as compared with the check plots. This increase can doubtless be explained in part on the ground that over 90 per cent of the tubers on the check plots were very severely scabbed, necessarily resulting in considerable decrease in weight. The severity of the attack is plainly evident from the appended photographs. These photographs are made up to show the relative proportion of clean, salable and unsalable scabby tubers from the various plots.

With both the uninoculated and the inoculated sulfur there was a marked decrease in unsalable scabby potatoes, amounting to 42.4 per cent for the former and 69.2 per cent for the latter. It is apparent that the greatest de-

TABLE 1

Influence of applications of inoculated and uninoculated sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD PER ACRE	CLEAN TUBERS	SCABBY BUT SALABLE	UNSALEABLE SCABBY	pH VALUES
	<i>bushels</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
*Check.....	195.0	0	6.4	93.6	7.06
*600 lbs. uninoculated sulfur per acre.....	229.0	10.9	37.7	51.4	6.23
*600 lbs. inoculated sulfur per acre.....	249.4	46.9	28.7	24.4	5.60

* Average of 3 plots.

crease resulted from the use of the inoculated sulfur. The benefit derived from the use of the latter is more clearly brought out when the number of tubers free from scab is considered. On the untreated plots none were clean as compared with 10.9 per cent for the plots treated with the uninoculated and 46.9 per cent for those receiving the inoculated sulfur.

In the diagrams of figure 1 are shown the relation of the hydrogen-ion exponent to the per cent of clean, salable and unsalable scabby potatoes. The initial hydrogen-ion exponent of this soil was 6.8 as compared with 7.05 for the check plots at the time of harvesting. This difference may be accounted for by the fact that the latter figure represents the composite soil sample from the check plots while the former represents that of the entire experimental area. It is evident however, that little change in exponent values occurred during the growing season. It will be seen from the diagrams that the plots receiving the 600-pound application of uninoculated sulfur showed a decrease in exponent values amounting to 0.83 as compared with 1.45 for those receiving the inoculated sulfur. With each decrease in the value of the hydrogen-ion

exponents below that of the check plots, a corresponding decrease occurred in the per cent of scabby tubers.

Section B. In this part of the field the scab infection was not as severe as in section A but there was a uniform distribution of the scab organism, a

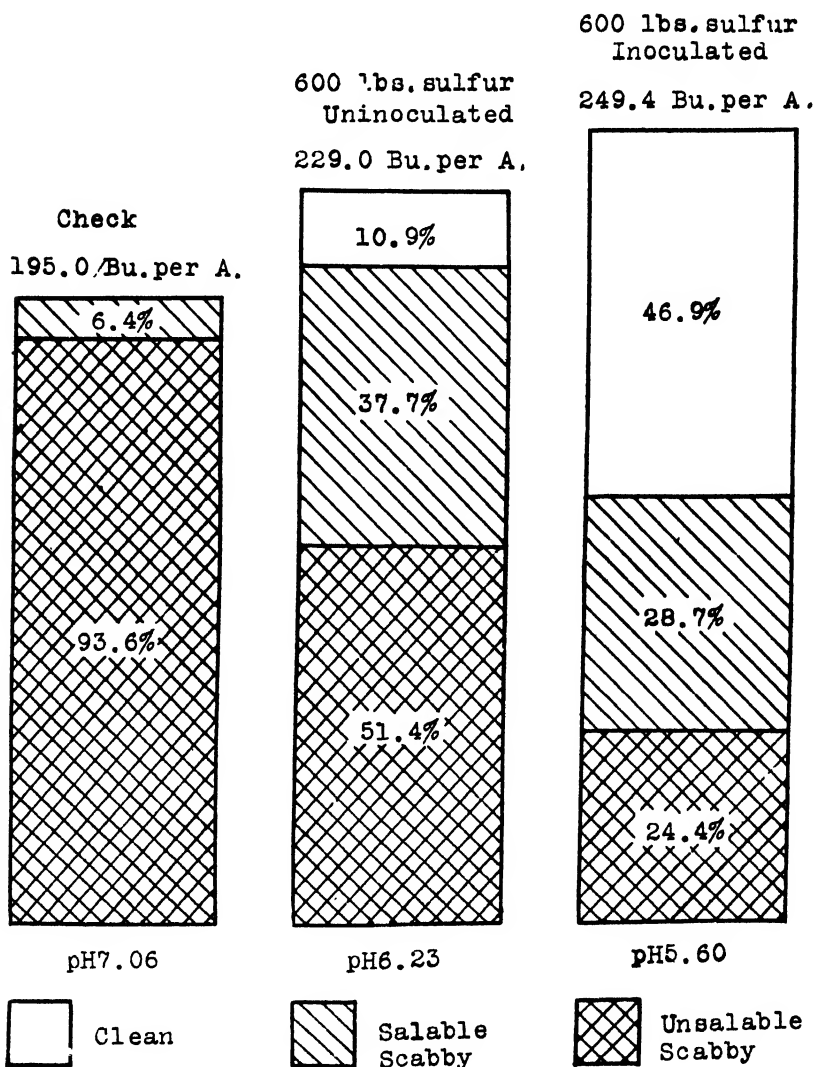


FIG. 1. DIAGRAMS SHOWING THE RELATION OF SULFUR TREATMENTS OF 600 POUNDS TO THE ACRE TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS

great part of the 1919 crop being severely scabbed. Applications of inoculated and uninoculated sulfur were made at the rate of 300 pounds per acre. The results are presented in table 2. The data of this table indicate a marked increase in yield for the treated over the check plots, amounting to 71.2 bushels

for the plots treated with the uninoculated and 105.6 bushels for those treated with the inoculated sulfur. It is doubtful if this increase can be ascribed to the sulfur treatment alone. In this part of the field the yield became progressively better away from the check plots so that the increases in yield noted may be attributed for the most part to soil differences.

In this as in the preceding section the plots receiving the sulfur treatments showed a lower percentage of scabby tubers than the untreated plots. The differences noted are by no means as great, however. Of the total yield from the untreated plots 58.3 per cent were unsalable scabby while the plots treated with the uninoculated and those receiving the inoculated sulfur gave a total yield of which 57.4 per cent and 29.4 per cent, respectively, were unsalable.

The relation of hydrogen-ion exponents to the per cent of clean, salable and unsalable scabby tubers is shown in the diagrams of figure 2. The initial exponent value of these soils was 6.8. At the time of harvesting, the average hydrogen-ion exponent values of soil samples taken from the check plots was 6.6 while the corresponding exponent values for the samples from the plots

TABLE 2

Influence of applications of inoculated and uninoculated sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD PER ACRE	CLEAN TUBERS	SCABBY BUT SALABLE	UNSALEABLE SCABBY	pH VALUES
	<i>bushels</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
*Check.....	183.1	9.4	32.3	58.3	6.6
*300 lbs. uninoculated sulfur per acre.....	254.3	13.1	29.5	57.4	6.3
*300 lbs. inoculated sulfur per acre.....	288.7	34.1	36.5	29.4	6.0

* Average of 3 plots.

treated with the uninoculated and those treated with the inoculated sulfur were 6.3 and 6.0, respectively. With the decrease in exponent values there was a corresponding decrease in the number of scabby tubers.

A comparison of the data included in tables 1 and 2 with their corresponding figures shows that the 600-pound applications gave much better control of scab than the 300-pound applications, this being true for both the inoculated and the uninoculated sulfur. This is apparent from the figures given in table 3, which represent the percentage of increase or decrease of clean, salable and unsalable scabby potatoes, as well as the differences in exponent values, of plots treated with inoculated and uninoculated sulfur as compared with their corresponding check plots. The data given here are in accord with those previously published (8) which showed that where the hydrogen-ion concentration of water extracts of soil samples taken before the sulfur applications were made was 5.8 or less, lighter applications (300 to 600 lbs.) gave approximately as good control of scab as heavier applications (700 to 1200 lbs.). Where the initial exponent was greater than 6.0 the heavier applications gave the better

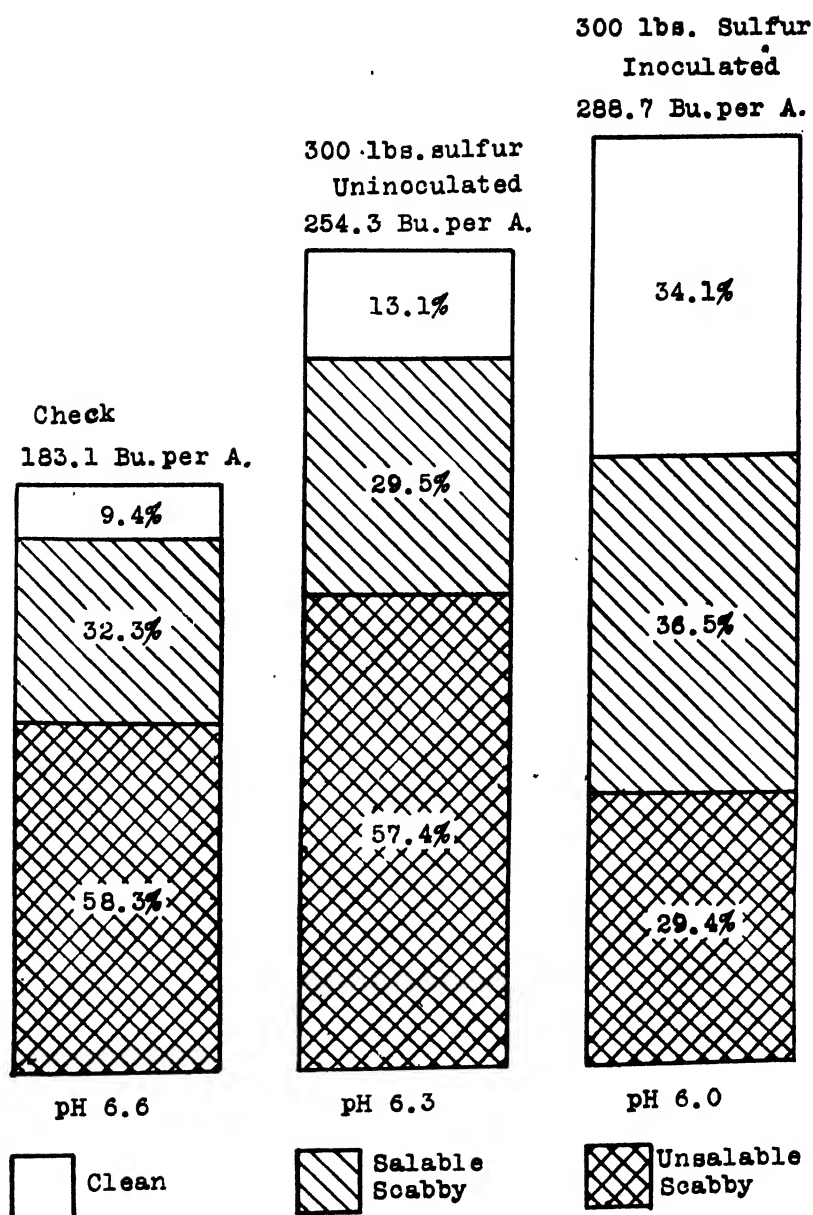


FIG. 2. DIAGRAMS SHOWING THE RELATION OF SULFUR TREATMENTS OF 300 POUNDS TO THE ACRE TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS

control. From the results here reported it is evident that the 300-pound application was not sufficient to produce the acidity necessary to inhibit the growth of the scab organism. It is also apparent that with an exponent value as great

as that indicated for the check plots in the first experiment, namely 7.03, the amount of sulfur used should be in excess of 600 pounds in order that a greater part of the crop be rendered free from scab.

It will be observed from table 3 that the 300 and 600-pound applications of inoculated sulfur gave a greater decrease in exponent values compared with that of the corresponding checks than did similar amounts of uninoculated sulfur. With the decrease in exponent values there was a decrease in the number of unsalable scabby tubers with a corresponding increase in the two classes made up of salable potatoes. It is interesting to note from the table that the decrease in exponent values indicated for the plots treated with 300 pounds of inoculated sulfur was nearly as large as for the plots receiving the 600-pound application of uninoculated sulfur. The latter showed a reduction of 42.2 per cent in the number of unsalable scabby tubers as compared with 28.9 per cent for the former, a difference of only 13.3 per cent in favor of the heavier application. Greater differences resulted from the use of 300 pounds of inoculated and uninoculated sulfur; the inoculated showed a decrease of 28.9 per cent in

TABLE 3

Percentage of increase or decrease of clean, salable and unsalable scabby potatoes and differences in exponent values of plots treated with inoculated and uninoculated sulfur as compared with their corresponding check plots

TREATMENT	CLEAN TUBERS	SALABLE SCABBY	UNSALEABLE SCABBY	DIFFER- ENCE IN pH VALUES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
300 lbs. uninoculated sulfur per acre.....	+3.7	-2.8	-0.9	-0.30
300 lbs. inoculated sulfur per acre.....	+24.7	+4.2	-28.9	-0.60
600 lbs. uninoculated sulfur per acre.....	+10.9	+31.3	-42.2	-0.83
600 lbs. inoculated sulfur per acre.....	+46.9	+22.3	-69.2	-1.46

the number of unsalable potatoes as compared with 0.9 per cent for the uninoculated sulfur, a difference of 28 per cent in favor of the inoculation. The plots treated with 600 pounds of inoculated sulfur showed 27 per cent fewer unsalable scabby potatoes than the plots receiving 600 pounds of uninoculated sulfur. From the data here presented it is evident that the presence of sulfolying organisms not only tends to make the oxidation of sulfur more certain but as a result of this fact smaller quantities of the inoculated sulfur may be used than of the uninoculated to obtain the same control of scab.

Experiment II

The soil on which this experiment was conducted is a Penn loam that had not been planted to potatoes for a number of years. To insure the presence of the scab organism in the soil scabby seed was planted. This fact may account for the very low yields recorded, less than 100 bushels per acre. In view of the fact, however, that the primary purpose of the work was to determine the effect of the sulfur on scab control the low yields cannot be considered

a serious fault since each plot was affected alike. In this experiment sulfur was applied at the rate of 900 pounds of the inoculated and the uninoculated per acre. The results are given in table 4.

On the basis of total yield the plots treated with the uninoculated sulfur showed a decrease of 17.8 bushels as compared with the check plots. The plots treated with the inoculated sulfur showed a slight increase. The soil on which this work was conducted was very uniform and while the crop showed considerable scab the infection was by no means as severe as in the first experiment, very few tubers being rendered unsalable. This, no doubt, accounts for the fact that the striking differences in yield noted in the other experiments were not obtained here. As in the preceding experiments, there was a marked reduction of unsalable scabby tubers on the plots treated with inoculated sulfur with a much smaller reduction in the plots treated with the uninoculated sulfur. The increase in the per cent of clean potatoes was likewise greater for the inoculated sulfur, amounting to 38.2 as compared with 26.1 for the uninoculated sulfur.

TABLE 4

Influence of applications of inoculated and uninoculated sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD PER ACRE	CLEAN TUBERS	SCABBY BUT SALABLE	UNSATABLE SCABBY	pH VALUES
	<i>bushels</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
*Check.....	78.0	43.2	34.4	22.4	6.6
*900 lbs. uninoculated sulfur per acre.....	60.2	69.3	15.4	15.3	4.6
*900 lbs. inoculated sulfur per acre.....	78.2	81.4	17.1	1.5	4.7

* Average of 4 plots.

The relation of the hydrogen-ion exponents of the soil samples from the plots in this experiment to the per cent of clean, salable and unsalable scabby tubers is shown in the diagrams of figure 3. Soil samples were taken of all the plots before the sulfur applications were made, the mean value of all the determinations being 6.61. The exponents of the check plots at the time of harvesting was 6.6. The values of the hydrogen-ion exponents of the soil samples corresponding to the two treatments show a considerable decrease over those of the check plots, being 4.6 for the plots receiving the uninoculated sulfur and 4.7 for those treated with the inoculated sulfur. While the exponent values for the two treatments are approximately identical, it will be seen from the diagrams that the number of unsalable tubers was reduced from 15.3 per cent recorded for the plots treated with uninoculated sulfur to 1.5 per cent, for those receiving the inoculated sulfur. On the basis of clean potatoes the latter showed an increase of 38.2 per cent over the checks as compared with 26.1 per cent for the uninoculated sulfur. It will be observed that the differences in scab control obtained in this experiment from the use of inoculated and uninoculated sulfur are by no means as great as in the first two experi-

ments reported. This may possibly be explained on the grounds that the plots in this experiment were smaller than in the others and their arrangement in the field was such that soil from one plot might have easily been carried to another in cultivation. In this way the plots treated with the uninoculated sulfur may have been inoculated at some time during the growing season. This would account for the fact that even with identical exponent values at digging time scab control was better on the plots receiving the inoculated sulfur than on those treated with the uninoculated sulfur. The former doubt-

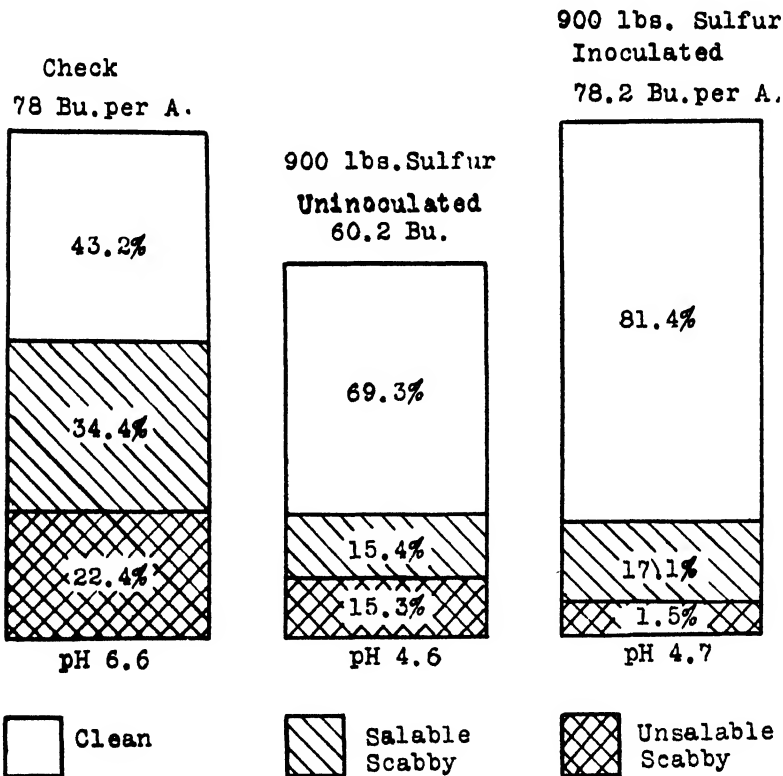


FIG. 3. DIAGRAMS SHOWING THE RELATION OF SULFUR TREATMENTS OF 900 POUNDS TO THE ACRE TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS

less oxidized earlier in the season bringing about a reduction in exponent values which resulted in the prevention of early infection. The latter, being inoculated at a later date, was not oxidized in time to prevent this early infection but was able to produce the same acidity by the time the crop was dug.

SUMMARY

1. The addition of sulfur to soil usually leads to an increase in soil acidity due largely to the oxidation of the sulfur by sulfifying micro-organisms. Where

these organisms are absent it is necessary that they be supplied in order that the sulfur be oxidized.

2. On the soils on which these experiments were conducted the use of sulfur inoculated with the sulfofying organisms gave better control of scab than similar amounts of uninoculated sulfur.

3. In addition to the difference in control the indications are that smaller amounts of inoculated than of uninoculated sulfur may be used to obtain the same results.

4. Hydrogen-ion exponent values of soil samples taken from plots treated with inoculated and from those treated with uninoculated sulfur were considerably lower than corresponding exponent values of soil samples taken from check plots. In most instances this increase in acidity was accompanied by a corresponding decrease in the number of unsalable scabby tubers.

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PLATE 1

FIG. 1. Tubers from check plots.

FIG. 2. Tubers from plots treated with 600 pounds uninoculated sulfur.

FIG. 3. Tubers from plots treated with 600 pounds inoculated sulfur.

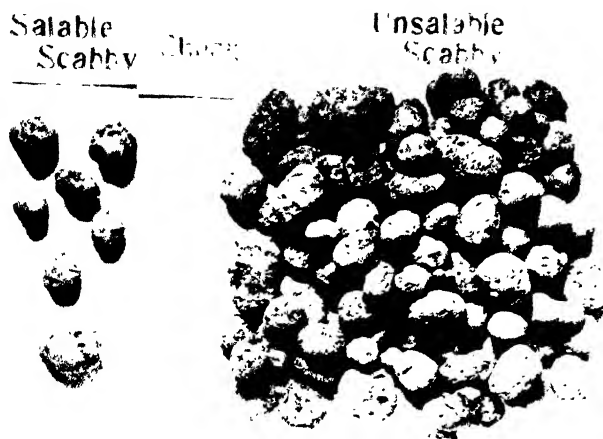


FIG. 1.

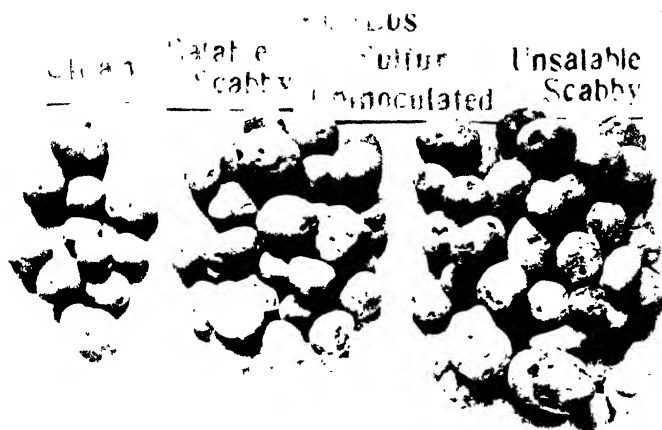
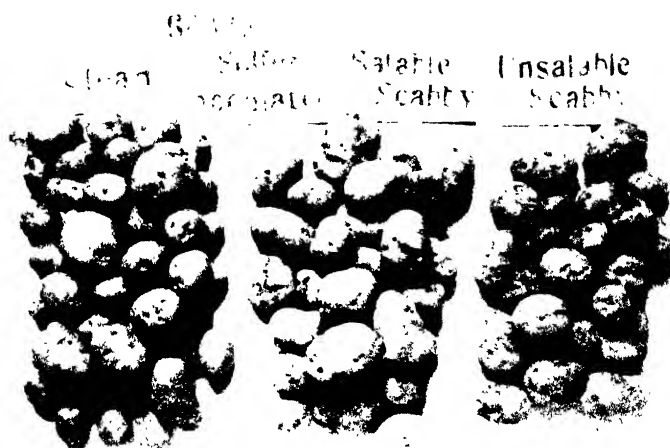


FIG. 2.



INOCULATED SULFUR AS A PLANT-FOOD SOLVENT¹

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Earlier communications by the senior author and his associates (1, 2, 3, 4, 5, 6, 7) contain a review of the literature, as well as original data on the subject of sulfur oxidation by microorganisms. In this paper there are recorded the results obtained with dry inoculated sulfur when the latter was applied alone or mixed with inert phosphatic or potassic materials.

The experiments discussed in this paper were carried on at New Brunswick, Whitesbog and Elmer, New Jersey. In the first of these, tests were made with uninoculated and inoculated sulfur as a possible means for increasing the availability to the crop of the phosphorus in ground phosphate rock, and of the potassium in greensand marl. In this case a portion of a field representing the soil type known as Sassafras loam was used. The field in question was neglected for many years prior to 1908. In the spring of that year it was plowed and levelled off by means of a road scraper. For a dozen years after this it was cropped with rye, oats and Canada field peas and soybeans. No fertilizer except acid phosphate, applied at the rate of 200 to 250 pounds per acre, was employed during that period.

In the spring of 1920 the field was laid out in $\frac{1}{16}$ -acre plots and utilized for two series of tests. The plots of series 1 received a uniform application of acid phosphate at the rate of 600 pounds per acre and of dried blood at the same rate. The additional treatments, on the acre basis, consisted of the following:

Plots 1-4	2000 lbs. greensand marl
Plots 5-8	500 lbs. uninoculated sulfur
Plots 9-12	2000 lbs. greensand marl and 500 lbs. uninoculated sulfur
Plots 13-16	500 lbs. inoculated sulfur
Plots 17-20	2000 lbs. greensand marl and 500 lbs. inoculated sulfur

The barley sown uniformly on May 17, came up unevenly. As the season progressed it was evident that the stand was much better on some of the plots than it was on the others. At the same time weeds sprang up in all of the space unoccupied by barley. Plots 14 and 15 were marked, more than the

¹ Paper No. 13, of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

others, by a thin stand of barley and a particularly luxuriant growth of weeds. Repeated observations made between June 1 and the end of July showed that the crop on plots 4, 8, 12, 16 and 20 was much better than it was on the corresponding plots that had received the same treatment. The differences were readily accounted for by the fact that the portion of the field on which these plots were located was of better quality than the remaining portion of the field. The observations also showed that the plots which had received applications of inoculated sulfur, or of inoculated sulfur mixed with greensand marl, had a better growth of both weeds and barley than the other plots. The plants on the former looked more vigorous and had a more intense green color.

The unusually favorable rainfall conditions stimulated the growth of the crop on all of the plots. They interfered later with the drying of the crop after it was harvested. For this reason there was a considerable shrinkage in the weight of the crop between the time of harvesting and that of taking the final weights about three weeks later. During this period the sheaves of barley and weeds were exposed to constant wetting by the heavy rainfall of August. The resulting shrinkage fell most heavily on the crops from the more heavy-yielding plots. Hence the final weights as recorded do not do full justice to the returns obtained from the plots that had received applications of inoculated sulfur, or of inoculated sulfur and greensand marl. The yields secured from the several plots, expressed as pounds of air-dry matter per acre, are shown in table 1.

TABLE 1
Crop yields per plot, treated with suljur and marl

PLOTS	WEIGHT OF CROPS				AVERAGE YIELD PER ACRE
	lbs.	lbs.	lbs.	lbs.	lbs.
1-4	26	16	24	24	2250
5-8	22	21	17	23	2100
9-12	21	21	17	19	1950
13-16	26	26	24	26	2550
17-20	25	25	22	26	2550

For the reasons already noted undue stress should not be laid on the air-dry weights recorded in table 1. Nevertheless the larger returns obtained from plots 13 to 16 and plots 17 to 20 are in accord with the superior appearance of the crops on these plots as it was noted during the growing season.

In another series of tests the $\frac{1}{16}$ -acre plots were treated as follows, calculated on the acre basis:

Plots 1-4	600 lbs. ground phosphate rock
Plots 5-8	200 lbs. uninoculated sulfur
Plots 9-12	600 lbs. phosphate rock and 200 lbs. uninoculated sulfur
Plots 13-16	200 lbs. inoculated sulfur
Plots 17-20	600 lbs. phosphate rock and 200 lbs. inoculated sulfur

Each plot received in addition to the above treatment 200 pounds each of ammonium sulfate and muriate of potash per acre.

As in the case of the sulfur-marl series, there were four plots of each treatment. The crop was also the same, namely, bearded barley. The observations as to weather conditions, weeds, etc., apply to this series as they do to series 1. The results secured are shown in table 2.

TABLE 2
Crop yields per plot, treated with sulfur and phosphate rock

PLOTS	WEIGHT OF CROPS				AVERAGE YIELD PER ACRE
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
1-4	26	26	42	30	3100
5-8	25	18	42	40	3125
9-12	19	27	48	37	3375
13-16	16	20	41	44	3025
17-20	35	25	47	49	3900

It will be observed that the combination of inoculated sulfur and ground phosphate rock gave decidedly better yields than any of the other treatments in this series. The weights as recorded bear out the observations made during the growing season, namely, that the inoculated sulfur and phosphate treatments produced darker-colored and more vigorous plants. The leaves of the barley plants on these plots were broader and larger and showed every sign of stimulated growth.

Without laying undue weight on the numerical results recorded in table 2, it may be concluded that the inoculated sulfur had apparently served the crop better than uninoculated sulfur when used together with ground phosphate rock. It would seem also that the combination of uninoculated sulfur and ground phosphate was superior to the ground phosphate rock when the latter was used alone.

The experiments at Whitesbog were carried out on a coarse sandy soil, poorly drained and acid in character. Plots 264 feet by 12 feet were laid out and corn was drilled in rows that were 4 feet apart. The treatment of the several plots on the acre-basis and the yields of green corn obtained are shown in table 3.

It appears from the results recorded in table 3 that the land used in this experiment was deficient in phosphorus, since the no-treatment plot as well as that receiving nitrogen, potash and sulfur, gave low yields. All of the treatments which included phosphorus gave marked increases in yield. The acid phosphate produced lower yields than did the rock phosphate used alone or in combination with sulfur. It also appears that the mixture of inoculated sulfur and rock phosphate (plot 6) gave better returns than the mixture of uninoculated sulfur and rock phosphate (plot 4). On the whole, the green weights as recorded are in agreement with the observations made during the

growing season. They seem to indicate that mixtures of inoculated sulfur and rock phosphate may supply economically and effectively both the phosphorus and sulfur requirements of crops.

Loam of very good quality was used for the experiments at Elmer. The land had been in grass until the midsummer of 1920. After plowing and fitting it was planted with Irish Cobbler potatoes, which were to be used ultimately for seed purposes. The land used in the experiment was rather uniform in

TABLE 3
Treatments and yields of corn in experiment at Whitesbog

PLOT NUMBER	TREATMENT PER ACRE	YIELD OF GREEN CORN PER ACRE
		<i>lbs.</i>
1	Nothing	9,657
2	75 lbs. nitrate of soda 75 lbs. dried blood 300 lbs. acid-phosphate 25 lbs. muriate of potash	14,202
3	75 lbs. nitrate of soda 75 lbs. dried blood 300 lbs. rock-phosphate 25 lbs. muriate of potash	18,450
4	75 lbs. nitrate of soda 75 lbs. dried blood 300 lbs. rock-phosphate 100 lbs. sulfur 25 lbs. muriate of potash	17,316
5	75 lbs. nitrate of soda 75 lbs. dried blood 100 lbs. sulfur 25 lbs. muriate of potash	10,710
6	75 lbs. nitrate of soda 75 lbs. dried blood 300 lbs. rock-phosphate 100 lbs. sulfur 25 lbs. muriate of potash and sulfofying bacteria	19,224

character, but the section represented by series 2 was of better quality than that of series 1. Each of the plots was equivalent to 1/45.2 acre in area. All of the plots except no. 1 and 7, which served as checks, received an application of nitrogen at the rate of 80 pounds per acre, and of potash at the rate of 100 pounds per acre. One-half of the nitrogen was derived from nitrate of soda and the other half from sulfate of ammonia. The potash was derived from muriate. The additional treatments consisted of 200 pounds per acre of

sulfur on plot 3. It will be observed therefore, that plots 2 and 3 received an application equivalent to that of a 4-0-5 fertilizer at the rate of 2000 pounds per acre; while plots 4, 5 and 6 received an application of the same fertilizer and in addition, equal weights of phosphatic material. There was, however, a difference in the amount and source of phosphorus used on plots 4, 5, and 6. For the first of these the phosphorus was supplied in 1000 pounds of acid-phosphate, for the second in 1000 pounds of rock-phosphate, while for plot 6 it was supplied in a mixture of 200 pounds of inoculated sulfur and 800 pounds of rock-phosphate. Hence the acid-phosphate supplied 160 pounds of total phosphoric acid. The corresponding amount for the rock-phosphate was 320 pounds; and for the bacsul-phosphate 256 pounds. The potatoes were planted

TABLE 4
Yield of potatoes on plots variously treated at Elmer

PLOT NUMBER	TREATMENT PER ACRE	YIELD PER ACRE			CALCULATED YIELD OF CHECKS	INCREASE PER ACRE OVER CHECKS
		Series 1	Series 2	Average		
		<i>bushels</i>	<i>bushels</i>	<i>bushels</i>	<i>bushels</i>	<i>bushels</i>
1	Check	101.8	113.8	107.8		
2	80 lbs. N, and 100 lbs. K ₂ O	115.2	122.4	118.8	105.2	13.6
3	80 lbs. N, 100 lbs. K ₂ O, and 200 lbs. sulfur	91.1	124.3	107.7	102.7	5.0
4	80 lbs. N, 100 lbs. K ₂ O, and 160 lbs. P ₂ O ₅ (acid-phosphate)	151.9	182.3	167.1	100.2	66.9
5	80 lbs. N, 100 lbs. K ₂ O, and 320 lbs. P ₂ O ₅ (rock-phosphate)	107.7	126.4	117.0	97.7	19.3
6	80 lbs. N, 100 lbs. K ₂ O, and 256 lbs. P ₂ O ₅ (bacsul-phosphate)	111.4	158.5	134.9	95.2	39.7
7	Check	87.4	98.0	92.7		

on August 5 and harvested November 5. The growing period corresponded, therefore, to the months of August, September and October, a time when the soil temperatures were gradually becoming lower and the conditions less favorable for the biological oxidation of the sulfur. The yields, expressed in terms of bushels per acre, are recorded in table 4.

It will be noted in the first place, that the soil responded to phosphorus treatment. The addition of phosphatic material increased in all cases the yield of the potato crop. Where acid-phosphate was used the increase was at the rate of 66.9 bushels per acre. Where rock-phosphate was used the increase was at the rate of 19.3 bushels per acre. Where bacsul-phosphate (the commercial name for mixtures of inoculated sulfur and ground phosphate-rock)

was used, the increase was at the rate of 39.7 bushels per acre. It would seem, therefore, that notwithstanding the well known preference of the potato crop for readily soluble phosphates, as well as the decreasingly favorable temperatures for the oxidation of the sulfur, the returns from bacsul-phosphate were far from unsatisfactory. At any rate, the data given in table 4 would justify the assumption that under suitable conditions bacsul-phosphate may prove to be an acceptable as well as an economical source of phosphorus for growing crops.

CONCLUSIONS

1. Inoculated sulfur seems to be more effective than uninoculated sulfur for rendering inert mineral plant-food accessible to growing crops.
2. Mixtures of inoculated sulfur and ground phosphate rock gave better returns than phosphate rock alone. Such mixtures may, after further experimentation, prove to be a satisfactory and economical source of available phosphorus.

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THE INFLUENCE OF IRON IN THE FORMS OF FERRIC PHOSPHATE AND FERROUS SULFATE UPON THE GROWTH OF WHEAT IN A NUTRIENT SOLUTION¹

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The use of iron in nutrient solutions for plants has not received the attention which the importance of this element as a necessary requirement for growth and development deserves. Many investigations bearing upon the general problem of the salt requirements of plants have been carried out and the development of our knowledge concerning the relation of green plants to the mineral elements essential to their growth has come largely through the use of nutrient solutions in these investigations. Many opportunities for constructive research may still be found in the general problem of the salt requirements of plants, and nutrient solutions will surely have to be employed in experiments dealing with this problem since the mineral elements essential to plant growth can not be supplied to the plants except in the form of aqueous solutions.

No nutrient solution is complete without iron. Many of the formulas of standard nutrient solutions which have been proposed for use in culture studies specify neither the kind (molecular composition, soluble or insoluble) nor the quantities of iron to be employed. The opinion has been pretty generally accepted that a trace of iron, in whatever form it may be found most convenient to supply it to the plants in culture solutions, is sufficient for the needs of growing green plants. However, investigations of the conditions under which plants can best obtain the iron necessary for their growth and development and studies of the different compounds of iron and the proper concentrations of these which are best adapted for absorption and assimilation by plants of different species under given sets of experimental conditions have been largely neglected within recent years.

The efficiency of the usual "trace" of iron when employed in culture solutions must certainly vary with the nature of the compound in which it is supplied to the plants, with the different species of plants, and with the nature of the solution in which it is employed. Corson and Bakke (1) have pointed out that the amount of iron used in nutrient solutions is probably of greater importance than is generally supposed. These authors have shown that ferrous

¹ Paper No. 11 of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Plant Physiology.

iron is less efficient than ferric iron when used in the forms of phosphates, and that the difference in the efficiency of these two forms of iron supplied to the plants in a nutrient solution is not nearly so pronounced with Canada field peas as it is with wheat. However, the plants were grown during a period of only 24 days, and as Tottingham and Beck (4) have pointed out the amount of reserve iron in the seed is probably one of the most important factors determining the response of plants to the supplies of iron during the early stages of development. Gile (2) has shown that the condition of the nutrient solution (whether acid, neutral, or alkaline) has a very marked influence upon the availability of iron to rice plants.

On the following pages are reported the results of a brief study of the influence of varying amounts of iron upon the growth and general appearance of spring wheat when supplied to the plants in a nutrient solution in the forms of the insoluble ferric phosphate and the soluble ferrous sulfate, these two forms of iron being chosen as adding no new ions to the nutrient solution employed.

In this study spring wheat of the "Marquis" variety was used and the general culture method adopted by Shive (3) was followed throughout. The seeds were sprouted on a germinating net and when about 4 cm. tall three selected seedlings for each culture were mounted in cork stoppers of the proper size and were transferred to the culture vessels which consisted of quart fruit jars of colorless glass. Shive's three-salt solution number R_5C_2 , having an osmotic concentration value of 1.75 atmospheres, was employed in all the cultures. Two series of ten cultures each were thus prepared. These will be designated series 1 and series 2 according as ferric phosphate and ferrous sulfate, respectively, were supplied as the source of iron. The culture solutions throughout each series differed only in the amounts of iron supplied while corresponding cultures of the two series differed only in the kind of iron used.

The ferric phosphate used in the culture solutions was prepared by precipitation from a dilute solution of ferric nitrate with a solution of mono-potassium phosphate. The precipitate was thoroughly washed, after which it was made into a suspension by the addition of distilled water and the iron was then determined by quantitative analysis of samples of the uniform suspension, and the total quantity adjusted, by the addition of distilled water, to contain 1 mgm. of iron in each cubic centimeter of this suspension which was used as a stock supply. Baker's "analyzed" ferrous sulfate crystals were used to supply iron in the soluble form. This form of soluble iron was chosen because it does not precipitate nearly so rapidly nor so completely from the culture solution here employed as do other forms of soluble iron such as ferric nitrate from which the iron is precipitated very rapidly and very completely as ferric phosphate. A stock solution of ferrous sulfate of such concentration that each cubic centimeter contained 1 mgm. of iron was freshly prepared each time just before being used in the culture solution. This was considered necessary since this salt in aqueous solution upon standing forms insoluble basic ferric sulfate as an oxidation product

The cultures of series 1 contained iron in the phosphate form varying in amounts from 0.01 mgm. to 5.0 mgm. of iron per liter of solution, while the corresponding cultures of series 2 were supplied with the same amounts of iron in the form of ferrous sulfate. The solutions were renewed regularly at intervals of from $3\frac{1}{2}$ to 4 days during a growth period of 90 days, at the end of which time the plants were well past the flowering stage. It was the plan of the experiment to grow the plants to maturity but unfortunately an attack of mildew (*Erysiphe germinis*) made it necessary to discontinue the cultures considerably before the seed had matured.

In table 1 are presented the numerical data of the experiment including the amounts of iron as such supplied to each culture in milligrams per liter of

TABLE 1

Total dry weights of wheat plants grown 90 days in Shive's three-salt solution No. R₃C₂ supplied with varying amounts of iron in the form of ferric phosphate and ferrous sulfate

CULTURE NUMBER	IRON PER LITER OF SOLUTION	SERIES I, FePO ₄		SERIES II, FeSO ₄	
		Total dry weight (3 plants)	Number of heads per culture	Total dry weight	Number of heads per culture
	mgm.	gm.		gm.	
1	0.01	4.55	0	10.95	0
2	0.10	5.75	0	14.65	0
3	0.25	8.20	0	15.95	1
4	0.50	10.35	0	12.80	1
5	0.75	12.05	0	27.40	9
6	1.00	14.90	0	18.75	4
7	1.50	16.25	0	19.15	2
8	2.00	20.50*	0	23.50	2
9	3.00	18.00	0	20.90	3
10	5.00	16.85	1	18.45	4
Check	0.00	8.40	0		

* Two plants only, calculated for three.

nutrient solution, the total dry weights of tops and roots, and the number of heads produced by the plants of each culture.

During the fourth week of the growth period the plants of series 1, in the cultures having the lowest amounts of iron, began to show the yellowish color in the leaves which is characteristic of plants suffering from an insufficient supply of iron. This chlorotic and general unhealthy condition of the plants gradually spread as time went on until the plants of all the cultures were affected, although it was at no time markedly pronounced in the plants of the culture containing the highest amount of iron. There was a uniform decline in the severity of the chlorotic condition of the plants in passing from the culture containing the lowest to that containing the highest amount of iron. In this series only a single head was produced, this occurring in the culture having the highest amount of iron.

The condition of the cultures in series 2 presents a sharp contrast to that of the cultures in series 1. The plants of all the cultures in series 2, except those in cultures 1 and 2, appeared healthy and vigorous throughout the entire growth period, aside from the attack of mildew which occurred shortly before the time of harvesting. The plants in cultures 1 and 2 which contained very small amounts of iron were chlorotic and produced no heads. All the other

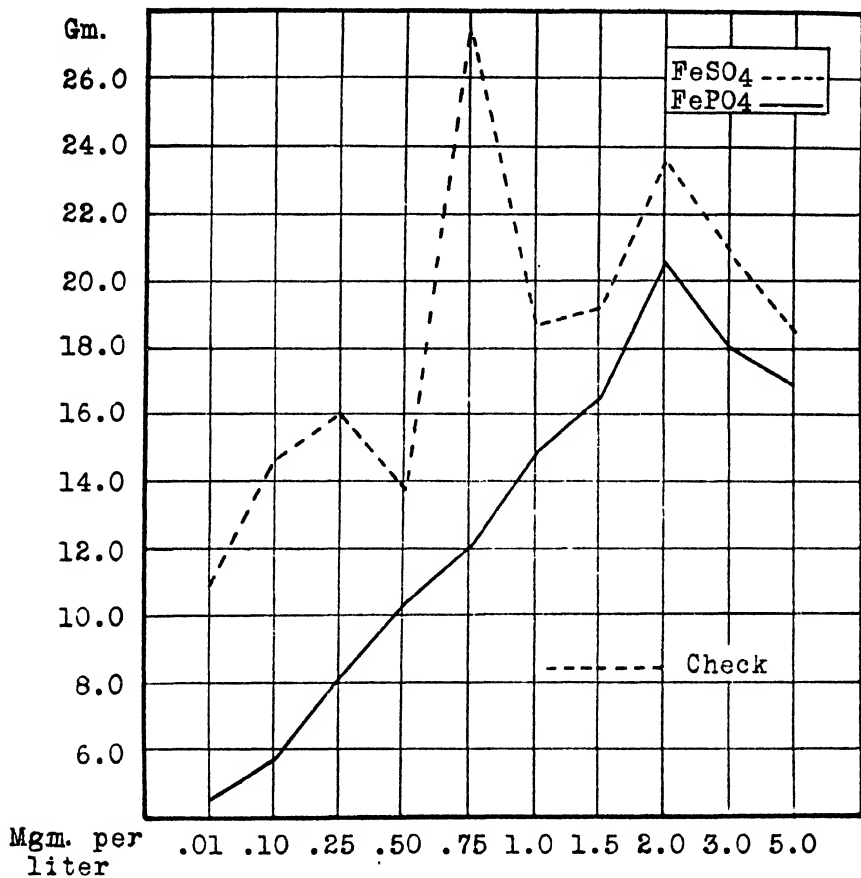


FIG. 1. TOTAL DRY WEIGHTS OF WHEAT PLANTS GROWN IN A NUTRIENT SOLUTION SUPPLIED WITH VARYING AMOUNTS OF IRON IN THE FORM OF FERRIC PHOSPHATE AND FERROUS SULFATE

cultures in this series produced heads the great majority of which were large and well formed. The general condition of cultures from each series about a week before the time of harvesting is shown photographically in plate I. These are corresponding cultures of the two series each supplied with the same amount of iron (0.75 mgm. per liter of solution), the one on the left receiving its iron in the form of ferrous sulfate, the one on the right in the form of ferric phosphate.

The total dry weight yields from the cultures of the two series as given in table 1, are shown graphically in figure 1. The dry weights as ordinates are here plotted against the amounts of iron in milligrams as abscissas. It will be observed that the yields from series 2 are much higher throughout than are those from series 1. The graphs of both series show a marked increase in yield with an increase in the amounts of iron supplied to the solutions up to an optimum which is not the same in both series. The maximum yield in series 1 was produced by the culture supplied with 2 mgm. of iron in the form of ferric phosphate while the maximum yield in series 2 was produced in the culture containing less than half this amount of iron in the form of ferrous sulfate. Both series show a marked decline in yields from the cultures containing more than 2 mgm. of iron per liter of solution. In the cultures of series 2 this may be the result of a toxic influence of too high concentrations of the soluble ferrous sulfate, although the general appearance of the plants in these cultures did not indicate this. On the other hand, the plants in the corresponding cultures of series 1, judging from the color of the leaves and the general appearance of the plants, suffered from an inadequate supply of iron, but the cause of the decline in yields is not clear. That the yellowish appearance of the leaves of the plants in the cultures of this series which were supplied with the highest amounts of iron was caused by the inability of the plants to secure the iron necessary for normal growth from the insoluble phosphate, was clearly demonstrated by the fact that duplicate cultures from this series when supplied with equivalent amounts of iron in the form of the soluble ferrous sulfate regained the normal green color of healthy plants in the course of two or three days.

It is thus clear that in the nutrient solution here employed iron in the form of ferric phosphate is very slowly and difficultly available to wheat plants even when supplied in relatively large quantities. Ferrous sulfate, on the other hand, appears to be readily available to these plants but is evidently somewhat toxic in the highest concentration used.

From the above considerations it appears that the insoluble ferric phosphate is not suitable for use with spring wheat in the culture solution here employed if the plants are to be grown beyond the stage of development when the reserve iron in the seed is no longer adequate to supply the needs of the plants. Ferrous sulfate, on the other hand, gave most excellent results when supplied to the culture solution here used in quantities of 0.75 to 3.0 mgm. of iron per liter of solution.

The limited experimental evidence here presented is only preliminary to a much more extended and complete study and does not justify the drawing of broad or definite conclusions, but the important generalizations touched upon will surely warrant more thorough investigation.

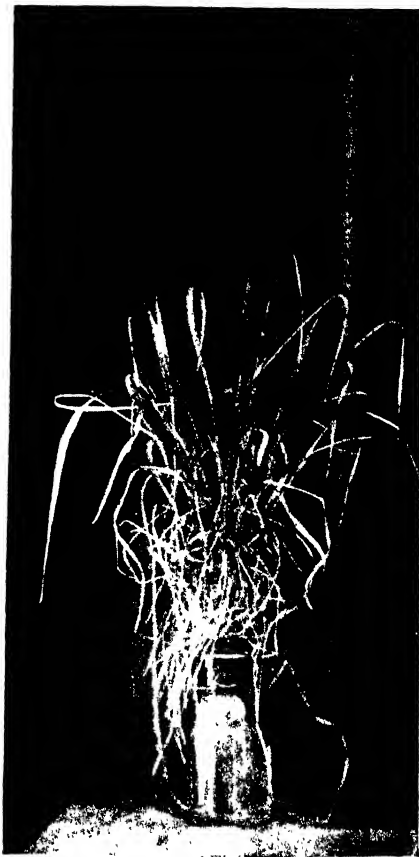
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PLATE 1

EFFECT OF DIFFERENT FORMS OF IRON ON THE WHEAT PLANT

The culture on the left received 0.75 mgm. of iron per liter of solution in the form of ferrous sulfate; the culture on the right received an equivalent amount of iron in the form of ferric phosphate



NITROGEN IN THE RAINWATER AT ITHACA, NEW YORK

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Since the middle of the Eighteenth Century it has been known that rain-water and snow contain nitrogen. The knowledge of this fact and the prime importance of nitrogen in agricultural practice have resulted in considerable work having been done to determine the amount of this element which the soil may receive from time to time through these agencies. As a rather full review of the literature on this subject is presented by Miller (7), in a report published in 1905 on the quantity of nitrogen and chlorine in the rainwater collected at Rothamsted, England, with one exception the writer will review only some of the articles which have appeared since that time.

Russell and Richards (8) have reported on the amount and composition of the rain falling at Rothamsted for a period of 28 years. It was found that, with an average annual rainfall of 28.82 inches, the ammoniacal nitrogen content was, on the average, 2.64 pounds per acre annually, while the nitric nitrogen was about one-half as much, or 1.33 pounds per acre. Monthly examinations of rainwater composed of daily collections showed the nitric nitrogen to vary only slightly from month to month, while the ammoniacal nitrogen was found to be highest during May, June, July and August and lowest during January, February, March and April. The yearly and monthly fluctuations in pounds per acre for both forms of nitrogen in the rainwater were generally in the same direction as were those for the rainfall, although this was not always the case. Since a close relationship was found between the amounts of ammoniacal and nitric nitrogen, the authors suggest a common origin or the conversion of ammoniacal forms into nitric forms. In addition to the ammoniacal and nitric nitrogen a yearly average of 1.35 pounds per acre of organic nitrogen was reported to be present in the rainwater. The sources of nitrogen are believed to be the sea, city pollution and the soil, the latter being an important source as the ammonia content of the rainwater is high and low at periods corresponding to high and low biochemical activity in the soil.

Crowther and Ruston (1) made analyses of rain collected for a period of 3 years at Garforth, England, and for a period of 12 months at different stations in the city of Leeds, England. An average annual rainfall of 26.95 inches at Garforth was found to contain 6.43 pounds of ammoniacal nitrogen and 1.93 pounds of nitric nitrogen to the acre. At Leeds the quantity of nitrogen in the rainwater varied at the different stations, ranging from 7.8 pounds per

acre to 18.4 pounds. In all cases the nitrogen was present largely in the form of ammonia or ammonium compounds, and it was only at stations some distance from the center of the city that nitrates could be detected at all. Organic nitrogen was found most abundant in the industrial area. The high nitrogen content of the rainwater collected at both Garforth and Leeds is attributed to the fact that they are situated in a large industrial center where the atmosphere is rich in impurities, notably ammonia.

With an annual rainfall of 32.55 inches at Flahult, Sweden (2) for the year 1909, von Feilitzen and Lugner found the rainwater to contain 3.32 pounds of ammoniacal nitrogen and 1.30 pounds of nitric nitrogen per acre. Their results show the nitrogen to vary considerably from month to month but the tendency is for both forms to be highest during the summer months.

Hudig (3) reports that an average yearly rainfall of 27.6 inches in the Province of Groningen, Netherlands, supplied an acre of soil with 4.54 pounds of ammoniacal nitrogen and 1.46 pounds of nitric nitrogen. It is stated that the amount of atmospheric nitrogen in the rainwater depends not only on the quantity of rain but on other meteorological conditions.

The monthly analyses of the rainwater collected for a period of 2 years at Bloemfontein and Durban in the Union of South Africa, reported by Juritz (4), show the summer rains to contain more nitrogen than the winter rains and the average amounts of nitrogen brought down yearly on the acre basis as ammonia and nitrates to be 4.02 pounds and 1.39 pounds, respectively.

Shipley (9) has reported the quantity of ammoniacal and nitrous nitrogen in the rainwater of Southwestern Alaska near Kashvik Bay during a rainy period in August, 1917. Ammonia was found to be almost entirely absent in the rainwater, which the author states is in striking contrast with the amount found at a similar latitude in Europe. Nitrous nitrogen was reported present in every sample of rainwater, with one exception, but the amount was always exceedingly small. No determinations were made for nitrate nitrogen.

Shutt and Dorrance (10) report the nitrogen compounds found in rainwater at Ottawa, Canada, for a period covering 10 years, during which time 897 samples were analyzed, 616 of which were rain and 281 of which were snow. Because of the greater solvent action of rain it was found to be much richer in nitrogen than was snow. With an average yearly precipitation of 23.39 inches for the decade, the soil received an annual supply of 6.58 pounds of nitrogen to the acre. Of this amount 3.41 pounds were in the form of free ammonia, 1.01 pounds in the form of albuminoid ammonia, and 2.16 pounds in the form of nitrates and nitrites. The writers point out that if the availability of this nitrogen is assumed to be equal to that in the more soluble nitrogenous fertilizers, the soil received during the 10 years reported an application of nitrogen equivalent to 440 pounds of sodium nitrate per acre. The rainwater contained its largest amount of nitrogen in August. This was true for all the forms of nitrogen studied except during September and October, 1908, when the free ammonia was unusually high on account of smoke contamination from

bush fires. Free ammonia gave the widest variation from month to month while albuminoid ammonia remained the most constant. Nitrate nitrogen was not found to be increased in the rainwater by electrical storms.

Several reports have been made on the nitrogen contained in rain and snow for short periods at Mt. Vernon, Iowa. For the period beginning January 12, 1910, and continuing for one year with the exception of the months of May, June, July and August, Knight (5) found in 17 samples of rain and snow, representing 6.81 inches of rainfall, nitrogen equivalent to 13.71 pounds per acre. Of this amount 2.04 pounds were in the form of free ammonia, 1.50 pounds in the form of albuminoid ammonia and 10.17 pounds in the form of nitrites and

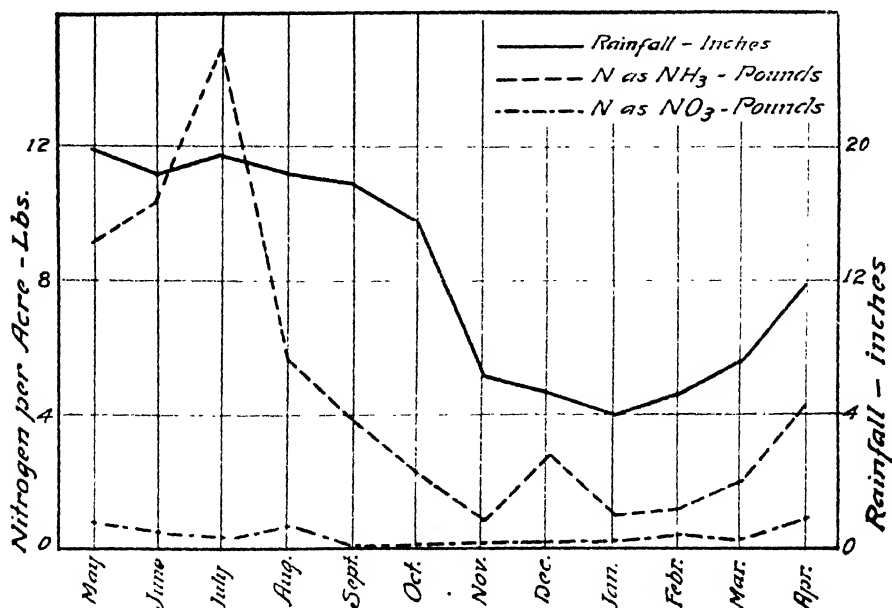


FIG. 1. RELATION OF NITROGEN TO RAINFALL
Period: May 1, 1915, to May 1, 1920

nitrites. These values for the different forms of nitrogen are quite different from those reported by Trieschmann (12) from the same place for the period between October 1, 1918 and June 15, 1919, when 46 samples of rain and snow representing a rainfall of 22.25 inches contained 5.28 pounds of nitrogen to the acre, 2.05 pounds being present in the rainwater as free ammonia, 1.84 pounds as albuminoid ammonia, 1.29 pounds as nitrate nitrogen and 0.09 pound as nitrite nitrogen. The latter author states that high winds and electrical discharges were not found to increase the quantity of nitrogen in the rainwater, that the total quantity of nitrogen found depends largely on the amount of rainfall, although an examination of the nitrogen supplied to the acre by each of the 46 precipitations showed a remarkable uniformity, and that rain was found to be richer in nitrogen than snow. While Knight found free ammonia

to be greater in rain than in snow he reports the latter, on the average, to contain more nitrites and nitrates.

The nitrate nitrogen found in the rainwater at Mt. Vernon for the first period mentioned above is exceedingly large and the relation of ammoniacal to nitrate nitrogen quite unusual. Miller (7) presents in tabular form the amounts of ammonia and nitric nitrogen found to be present in the rainwater of temperate and tropical countries. In the non-tropical rain where analyses were continued for at least four years, Lincoln, New Zealand, was the only one of ten different places where the nitric nitrogen was found to be in excess of the ammoniacal nitrogen. This relation was due to an unusually small amount of ammonia rather than to a high nitric nitrogen content. The rainwater of the tropical countries listed contained more nitrogen as ammonia than as nitric nitrogen, except that falling in British Guiana and Barbados. In neither place, however, was the yearly quantity of nitric nitrogen greater than 3.88 pounds to the acre.

As data on the nitrogen in the rain falling in this country are quite limited the report of Tracy (11) on the quantity of this constituent in the rainwater at Agricultural College, Mississippi, for the years 1894 and 1895 is here mentioned. With an average yearly rainfall of 44.11 inches for the two years the soil is reported to have received per acre, annually, 2.35 pounds of ammoniacal nitrogen and 0.73 pound of nitrate nitrogen.

While the total nitrogen found in the rainwater varies considerably, certain observations have been made which seem to be quite generally true throughout the world. The ammoniacal nitrogen is found to be in excess of the nitrate nitrogen. The former fluctuates from year to year and from month to month, while the latter remains more constant throughout the year, being slightly higher during the summer season. Summer rains have been found to contain more nitrogen than winter rains and snow less nitrogen than rainwater. The precipitation determines very largely the total amount of nitrogen supplied to the soil. Wind, electrical discharges and other meteorological conditions appear to have but little effect on the quantity of oxidized nitrogen in the rainwater. These latter generalizations, however, are not in accord with the findings of Masson (6), working in Australia, who reports that the total oxidized nitrogen found in the rainwater accompanying a storm depends upon the type of weather and is practically independent of the amount of rainfall, and that the concentration of oxidized nitrogen varies inversely with the amount of precipitation.

In order to determine the quantity of nitrogen in the precipitation at Ithaca, New York, analyses of the rainwater have been made for a number of years and the results that have been obtained are herein reported.

COLLECTIONS OF RAINWATER

Collections of the rainwater were begun in August, 1914, and are continued at the present time. Monthly examinations have been made for ammoniacal

and nitrate nitrogen and from the quantity of water collected by months, the yearly precipitations have been determined. The rain and snow are collected in a metal rain-gauge 8 inches in diameter which stands about 9 feet from the ground and which is protected from birds by a movable frame projecting a little above and beyond the top of the gauge. The latter is located in a field near which there are no factories and as this area is almost continually under cultivation or covered with snow the air is comparatively free from smoke and dust. A railroad running about one-half mile to the south and a small heating plant located about one-half mile to the west of the field, undoubtedly, at certain times, contaminate the air to some extent.

The rain-gauge is emptied after each rain or snow and its contents stored in glass bottles, which contain a few drops of mercuric chloride solution, until the first of each month when the water is measured, filtered when necessary to remove any insects or wind blown material and the nitrogen determined.

ANALYSIS OF THE RAINWATER

The samples of rainwater are analyzed for ammoniacal nitrogen by the Nessler reaction as used in water analysis and for nitrate nitrogen by the phenol-disulfonic-acid method. Organic nitrogen is not determined. When present in the rainwater it is believed to be largely due to contamination from foreign particles existing temporarily in the atmosphere near the surface of the earth and a more accurate value for the nitrogen actually brought down by the rain is probably obtained by disregarding it.

NITROGEN IN THE RAINWATER

The results reported are for the ammoniacal and nitrate nitrogen content of the rainwater from August 1, 1914 to May 1, 1920. As the nitrogen in the rainwater for May, June and July for the experimental year 1914-1915 was not determined, this discussion will be confined to the nitrogen in the rainwater for a period of five years beginning May 1, 1915 and ending May 1, 1920.

The quantity of nitrogen found in the rainwater for each month of the several years is presented in tables 1 to 6, inclusive, together with the corresponding monthly precipitation. The data given in table 1 cover a period of only 9 months, and as the rainfall is comparatively large for the three months not tabulated these data are not included in table 7 in which the results for the period mentioned above are brought together. It may be seen from this latter table that with an average rainfall of 29.31 inches, the soil received an average yearly supply of 12.51 pounds of nitrogen to the acre, 11.5 pounds of which were ammoniacal nitrogen and 1.01 pounds of which were nitrate nitrogen. This value for the ammoniacal nitrogen is much greater than the values generally ascribed to it. At Rothamsted, England, where the average annual rainfall for 28 years was slightly less than the one reported above, only 2.64

pounds of ammoniacal nitrogen were found. The value for the nitrate nitrogen is in close agreement with the values found by other workers.

The articles reviewed by Miller (7) show the total nitrogen found in the rainwater of thirty different localities to vary from 19.91 pounds to 1.63 pounds per acre yearly. The largest amount was found in the rainwater at Proskau, Germany, while the smallest quantity was found in that of Lincoln, New Zealand. The total nitrogen found in the precipitation for the five years reported at Ithaca, as may be seen in table 7, lies about midway between these two extremes.

It is quite evident from the accompanying figure, in which the results given in table 7 are shown graphically, that the ammoniacal nitrogen fluctuates from

TABLE 1
Nitrogen in the rainwater between August 1, 1914, and May 1, 1915

MONTH COLLECTED	RAINFALL	N AS NH ₃	N AS NO ₃	N PER ACRE
	<i>ins.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>lbs.</i>
August, 1914.....	5.58	3.82	0.38	1.15
September.....	1.98	0.96	0.07	0.28
October.....	1.13	0.61	0.08	0.19
November.....	0.98	1.13	none	0.31
December.....	1.94	1.17	none	0.32
January, 1915.....	5.20	2.10	none	0.58
February.....	1.19	1.11	none	0.31
March.....	0.27	0.38	none	0.10
April.....	0.73	5.83	0.43	1.72
Total 9 months.....	19.00	17.11	0.96	4.96

TABLE 2
Nitrogen in the rainwater between May 1, 1915, and May 1, 1916

MONTH COLLECTED	RAINFALL	N AS NH ₃	N AS NO ₃	N PER ACRE
	<i>ins.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>lbs.</i>
May, 1915.....	2.31	6.04	0.73	1.86
June.....	2.85	9.12	none	2.51
July.....	6.34	20.28	none	5.58
August.....	3.24	6.72	none	1.84
September.....	2.39	6.89	none	1.89
October.....	3.83	1.91	none	0.53
November.....	1.01	0.47	0.16	0.17
December.....	2.07	1.49	0.60	0.57
January, 1916.....	0.17	0.22	0.17	0.11
February.....	2.31	1.05	0.43	0.41
March.....	1.47	1.08	none	0.30
April.....	2.33	4.36	0.68	1.39
Total for year.....	30.32	59.63	2.77	17.16

TABLE 3
Nitrogen in the rainwater between May 1, 1916, and May 1, 1917

MONTH COLLECTED	RAINFALL	N AS NH ₃	N AS NO ₃	N PER ACRE
	<i>ins.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>lbs.</i>
May, 1916.....	3.72	12.39	none	3.40
June.....	3.35	12.21	none	3.36
July.....	2.67	14.88	none	4.09
August.....	1.77	3.60	0.22	1.05
September.....	5.71	3.47	none	0.95
October.....	1.45	2.31	none	0.64
November.....	1.45	0.93	0.54	0.40
December.....	1.00	7.25	0.30	2.08
January, 1917.....	1.12	1.97	0.33	0.63
February.....	0.27	0.79	0.14	0.26
March.....	1.08	2.66	0.84	0.96
April.....	1.81	5.79	1.28	1.94
Total for year.....	25.40	68.25	3.65	19.76

TABLE 4
Nitrogen in the rainwater between May 1, 1917, and May 1, 1918

MONTH COLLECTED	RAINFALL	N AS NH ₃	N AS NO ₃	N PER ACRE
	<i>ins.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>lbs.</i>
May, 1917.....	3.98	12.70	0.89	3.74
June.....	7.11	14.79	1.46	4.48
July.....	2.94	15.04	0.77	4.35
August.....	8.30	7.96	1.23	2.53
September.....	2.31	0.92	none	0.25
October.....	4.66	2.17	0.55	0.75
November.....	0.41	0.80	0.09	0.24
December.....	0.68	0.18	none	0.05
January, 1918.....	0.68	0.81	0.10	0.25
February.....	0.99	1.08	0.54	0.45
March.....	1.56	0.67	none	0.18
April.....	2.88	1.47	0.36	0.50
Total for year.....	36.50	58.59	5.99	17.77

TABLE 5
Nitrogen in the rainwater between May 1, 1918, and May 1, 1919

MONTH COLLECTED	RAINFALL	N AS NH ₃	N AS NO ₃	N PER ACRE
	<i>ins.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>lbs.</i>
May, 1918.....	5.06	0.71	0.75	0.40
June.....	2.98	0.82	none	0.23
July.....	4.16	3.45	none	0.95
August.....	3.48	1.84	0.48	0.64
September.....	5.73	1.65	none	0.45
October.....	2.50	0.54	0.37	0.25
November.....	0.89	0.28	0.20	0.13
December.....	0.55	0.26	none	0.07
January, 1919.....	0.79	0.42	0.47	0.24
February.....	0.70	0.34	0.30	0.18
March.....	1.52	0.97	none	0.27
April.....	1.93	0.84	0.50	0.37
Total for year.....	30.29	12.12	3.07	4.18

TABLE 6
Nitrogen in the rainwater between May 1, 1919, and May 1, 1920

MONTH COLLECTED	RAINFALL	N AS NH ₃	N AS NO ₃	N PER ACRE
	<i>ins.</i>	<i>mgm.</i>	<i>mgm</i>	<i>lbs.</i>
May, 1919.....	4.82	1.54	0.63	0.60
June.....	1.82	0.70	0.37	0.30
July.....	3.27	0.88	0.70	0.43
August.....	1.31	0.22	0.49	0.20
September.....	1.47	0.56	none	0.15
October.....	2.82	0.72	none	0.20
November.....	2.22	0.50	none	0.14
December.....	0.76	0.49	none	0.13
January, 1920.....	0.92	0.19	none	0.05
February.....	0.69	0.77	0.13	0.24
March.....	1.37	1.67	0.26	0.53
April.....	2.56	2.29	0.29	0.71
Total for year.....	24.03	10.53	2.87	3.68

TABLE 7
Nitrogen in the rainwater between May 1, 1915, and May 1, 1920

MONTH COLLECTED	RAINFALL	N AS NH ₃ PER ACRE	N AS NO ₃ PER ACRE	TOTAL N PER ACRE
	<i>ins.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
May.....	19.89	9.18	0.82	10.00
June.....	18.11	10.37	0.51	10.88
July.....	19.38	15.00	0.40	15.40
August.....	18.10	5.59	0.67	6.26
September.....	17.61	3.70	none	3.70
October.....	15.26	2.11	0.26	2.37
November.....	5.98	0.82	0.27	1.09
December.....	5.06	2.66	0.24	2.90
January.....	3.68	0.99	0.29	1.28
February.....	4.96	1.11	0.43	1.54
March.....	7.00	1.94	0.30	2.24
April.....	11.51	4.06	0.85	4.91
Total.....	146.54	57.53	5.04	62.57
Yearly average.....	29.31	11.50	1.01	12.51

month to month while the nitrate nitrogen remains fairly constant and that the total nitrogen depends very largely on the amount of rainfall. While it is generally true that with a large precipitation the ammoniacal nitrogen is correspondingly large, there is a consistent falling off in this form of nitrogen with the month of August, and it continues to decrease in spite of the usual heavy rainfall during this and the following two months. This decrease in the amount of ammoniacal nitrogen may be due to the fact that the heavy rains of the late spring and early summer have washed the greater portion of the ammonia from the atmosphere. It has been observed by Shutt and Dor-

rance (10) that continued rains free the atmosphere of nitrogen and that a short shower usually shows higher nitrogen concentration than a longer one. An inspection of tables 2 to 6, inclusive, will show this falling off in the ammoniacal nitrogen to occur for each of the five years reported.

As the rainwater was analyzed only monthly it is impossible to state the effect of electrical discharges on its nitrate nitrogen content. Table 7 shows the nitrates to be somewhat higher in the rainwater of the spring and summer months than they are during the remainder of the year and since they are found to be highest during the time of the most frequent electrical storms it is possible that some of the nitrate nitrogen present is the result of the oxidation of other forms of nitrogen existing in the atmosphere. This table also shows very much more nitrogen to be brought down in the summer rain than in the winter precipitation. The rain falling during May, June, July and August contained 67.88 per cent of the total nitrogen brought down in the rainwater between May 1, 1915, and May 1, 1920.

The relatively high amount of ammoniacal nitrogen found in the rainwater at Ithaca can not be attributed to contamination, as the air is unusually free of dust and smoke. The greatest source of contamination is probably from the soil particles blown about in the immediate vicinity of the rain-gauge. As previously stated a railroad and a heating plant, both of which are located about one-half mile from the rain-gauge undoubtedly increase the ammonia in the rainwater at certain times. These sources of contamination, however, could not account for the consistent differences in the amounts of ammonia in the rainwater for the months of July and August and in all probability furnish only a very small portion of the total quantity of this constituent found to be present in the yearly precipitations.

SUMMARY

With an average yearly rainfall of 29.31 inches between May 1, 1915, and May 1, 1920, the soil received annually 12.51 pounds of nitrogen to the acre. Of this amount 11.5 pounds was in the form of ammoniacal nitrogen and 1.01 pounds in the form of nitrate nitrogen.

The ammoniacal nitrogen was found to fluctuate from month to month and from year to year while the nitrate nitrogen remained more constant. The amount of total nitrogen in the rainwater was to a large extent dependent on the amount of rainfall, a high nitrogen content accompanying a correspondingly high precipitation.

The rainfall during the spring and summer months contained more nitrogen than the rain falling during the other two seasons of the year. The ammoniacal nitrogen decreased rather suddenly during August and continued low during September and October in spite of heavy rainfalls. This decrease was probably due to the atmosphere being washed comparatively free of ammonia by previous rains.

Electrical discharges did not increase the nitrate nitrogen content of the rainwater to any considerable extent.

The amount of ammoniacal nitrogen brought down in the rain falling at Ithaca, New York, is somewhat larger than that reported to be present in many parts of the world, while the nitrate nitrogen content is about the same.

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A SHORT TEST FOR EASILY SOLUBLE PHOSPHATE IN SOILS¹

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INTRODUCTION

A large amount of work has been done on the digestion of soils with weak solvents, especially acids, in attempts to separate the easily soluble plant-food constituents from the less-soluble portions. The one kind is generally called easily soluble, available or active, and the other dormant, non-available, or, preferably, potential plant-food. This has come about as a result of efforts made to devise somewhat arbitrary methods for measuring that portion of the plant-food in soils which can be utilized by crops at the present time as distinguished from the remainder which in all probability will become more slowly available in the future.

HISTORICAL

Numerous methods have been devised for the above purpose and frequently the results obtained by different forms of procedure do not agree; consequently, there has arisen considerable discussion among scientific workers interested in this subject as to the value of such methods for the purpose intended, which has divided them into two general groups. One comprises those who believe that, on account of it being impossible to duplicate soil conditions in the laboratory by the use of arbitrary chemical methods, no credence can be placed in work of this character. To support this they cite the fact that the results obtained by such methods often vary widely among themselves. There are also some among this class who not only take the position that these methods have no significance, but maintain that all chemical methods, including those for the estimation of total plant-food constituents, give no reliable data for indicating the productive capacity of soils. The other group, while conceding the above to be partly true, nevertheless contend that some of the methods, although they may have their limitations, do give results of a certain value when used by workers with sufficient experience who know how to interpret them in connection with other data that may be obtained. For this reason they continue to employ them in their work.

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It is not necessary to discuss the relative merits of these opposite views or to include a summary of all the work that has been done, as the references in the literature pertaining to this subject are very numerous. For this reason only those which refer to certain matters discussed here will be mentioned.

During the progress of the work given in tables 2 to 5, an article came to the attention of the writer describing some work by Russell and Prescott of the Rothamsted Experiment Station (5), who have made rather extensive experiments on the determination of phosphorus in weak acid digestions of soils. Their work has been confined to a study of this element and among other things includes its adsorption by the soil and the effect of time of digestion and different strengths of various acids on the results obtained. While their experiments are somewhat different from those employed in this work, it is of interest to note that their results show that a short digestion with some acids, especially nitric, not exceeding a certain strength, puts more phosphorus into solution than does one for a longer time. This they prove to be due to adsorption. On account of the different methods of procedure and the limited number of soils used in their work, the conclusions reached regarding the behavior of nitric acid have been only partly confirmed here. An excellent summary of some previous investigations on adsorption phenomena in soils is given by these writers and in a resumé by Prescott (4).

Of all the methods that have been proposed for the estimation of easily soluble constituents in soils, probably the most widely used in this country are those involving the digestion of the soil with weak acids, either 0.2*N* hydrochloric or nitric, for a limited time at a definite temperature. The former was first adopted by the Association of Official Agricultural Chemists as a provisional method and included in their Methods of Analysis (3, p. 18).

Probably the first work with 0.2*N* nitric acid as a solvent for plant-food in soils was done by Dr. A. M. Peter, at this station, and the results were so favorable that it was proposed for trial to the association. Later, a committee appointed for the purpose of editing and revising their methods of analysis recommended it as a tentative method to replace the weak hydrochloric digestion (1, p. 27). It should be stated, however, that the method as recommended differs materially in some respects from the procedure as carried on here.

The last committee appointed by the association for the revision of methods has failed, for some unaccountable reason, to include any method for the above separations; consequently none appears in the latest revised Methods of Analysis of the association (2).

EXPERIMENTAL

In the early part of 1916, the writer, while engaged in some work on the digestion of soils with 0.2*N* nitric acid and other weak solvents, observed that adsorption apparently had considerable influence on the results obtained,

especially in the phosphorus determinations. It was found at that time (in some unpublished work) that in the 0.2*N* nitric acid digestion, when equal volumes of the acid were used on different amounts of soil, the smaller weight of soil showed more phosphorus in solution after the digestion, in proportion to the quantity of soil used, than was to be expected.. An explanation which suggested itself at the time was that where the smaller mass was used, its adsorptive capacity was not satisfied under the conditions of the experiment, but when a larger amount of the same was in contact with the richer solution of phosphate, this requirement was more nearly met. It is possible, however, that the smaller mass of soil allows its particles to be exposed more completely to the relatively larger volume of acid and that this partly accounts for the discrepancies. The results obtained are given in table 1.

TABLE 1

Phosphorus dissolved by 1500 cc. of 0.2 N HNO₃ from different weights of soil, after 5 hours' digestion, being shaken every 30 minutes*

SOIL NUMBER	PHOSPHORUS OBTAINED FROM		RATIO
	7.5 gm. of soil	150 gm. of soil	
	gm.	gm.	1:20
56525	0.0003	0 0010	1:3.3
56526	0 0010	0 0059	1:5.9
56528	0.0008	0 0020	1;2.5
56530	0.0003	0.0009	1:3.0
14604	0 0327	0 4303	1:13.2
14606	0.0005	0.0026	1:5.2
56527	0.0003	0 0011	1:3.7
56529	0 0002	0 0005	1:2.5
56531	0.0003	0.0016	1:5.3
56532	0 0002	0 0004	1:2.0

* These digestions were made at room temperature, as were all others described in this paper.

A large excess of free nitric acid was present at the end of the digestion, in every experiment. That the solvent power of the acid for phosphate was not satisfied is apparent from the large amount of phosphorus taken up from soil no. 14604, as compared with that dissolved from the other soils. It should be borne in mind that the neutralizing power of our soils for 0.2*N* nitric acid, under the conditions of these experiments, is practically negligible except when carbonate is present. Assuming that the action was only that of an acid on the soil with no subsequent fixation of the dissolved phosphate, and that the soil surfaces exposed were somewhat in proportion to the amounts of soil used, the ratio of the phosphorus dissolved would be expected to be more comparable to the relative amounts of soil present. The ratio of the latter was constant (1:20) while that of the former varied considerably in individual soils, the maximum being 1:13.2 and the minimum 1:2. In other

words, the last ratio indicates in these particular soils where the larger amounts were used, only about one-tenth of the quantity of phosphorus in solution that would be expected, provided no adsorption or other interfering factors had intervened.

After the foregoing experiments were completed, attempts were made to determine if adsorption in soils was exerting any considerable influence on the amounts of plant-food constituents obtained in the weak acid digestion. In order to demonstrate this point, digestions were made of the same amounts of soil for short and longer periods of time and it was found that short digestions—for instance, 5 minutes—in 0.02*N* HNO₃, with shaking every minute, gave higher results with some soils as regards certain constituents, especially phosphorus, than the prescribed 5-hour period, with shaking every 30 minutes. In fact, in some soils, by placing them on folded filters, pouring the acid on and allowing it to filter immediately, the results compared favorably with the longer contact. This, however, did not hold true with all soils.

The above work comprises part of an investigation that is being carried on by the writer in an effort to obtain, if possible, more definite knowledge concerning the effect of weak acid digestions of our soils regarding certain constituents, namely phosphorus, potassium, calcium and silica. That such digestions, especially for short intervals of time, do have a certain value for indicating the productive capacity of a soil, is believed for the following reasons. First, the short, weak acid digestion largely eliminates the time action of the acid on the soil and this is one objection that has been raised against all methods of this character; second, the error due to adsorption by the soil, especially if it is of a gradual nature, is partly eliminated; third, the soil silicates are not appreciably attacked, as shown by the silica obtained in the digestion and finally a comparison of the short and the long digestions shows that about the same amount of calcium, a large bulk of the potassium and, in many cases, as much phosphorus is obtained in the former. The inference is that the amounts so obtained are more loosely held and in all probability are combined with the organic matter or humus portions of the soil rather than with the mineral silicates. As stated before, however, this work is being continued and a publication will probably be issued in the near future.

During the last few years the number of soils sent in by our farmers for tests as to their fertilizer requirements has been increasing to such an extent that short qualitative tests have been resorted to in answer to these demands. One of the tests generally made on such samples is for total phosphorus, for the reason that it is one of the most deficient elements in our soils. Unfortunately, however, the test for easily soluble phosphorus requires more time and quantitative work. Therefore it was thought that if some short test could be developed for finding out something about the amount of this element taken up in weak acid, it would be of some benefit in arriving at the need of the soil for soluble phosphate, when considered in connection with the total amount present.

As the short digestion gave as good results with a large number of soils as the longer, the following test, among others, was tried:

Ten grams of air-dried soil was added to 25 cc. of 0.2*N* HNO₃ and the whole shaken every minute for 5 minutes. The solution was filtered until clear into a $\frac{3}{4}$ by 6 inch test tube, 1 or 2 cc. of 60 per cent NH₄NO₃ solution was then added, and 5 cc. of ordinary molybdate solution as prescribed in the official method for fertilizers (3, p. 2). The contents of the test tube were heated to about 60°C., shaken several times and allowed to stand about 30 minutes at room temperature.

A possible objection may be raised against a method of this kind. Since it is only a qualitative test, how are the results obtained by it to be described to an inexperienced worker so that he can interpret them when made? In answer to this it might be stated that those given in the following tests were made by the writer without any previous experience and none have been changed or discarded.

The results marked "large," "fair," "moderate," "very moderate," etc., stand in decreasing order of the amount of ammonium phospho-molybdate obtained. These terms are only approximately defined and in some instances may be partly interchangeable without seriously vitiating the interpretation of the test. Although difficult to describe exactly, the term "moderate" indicates that the yellow precipitate, after settling by gentle tapping of the tube, occupied an area in the rounded tip from 0.5 to about 0.75 cm. in diameter.⁸ After a little experience no great difficulty will be found in the relative terms to be applied for purposes of comparison.

After the short tests were all made, the results were compared with the quantitative results from 5-hour digestions, so that no preconceived judgment might be formed about the terms to be applied. As stated above, none were changed or discarded.

The 0.2*N* HNO₃ digestion was made by digesting the soil in the proportions of 1 gm. of soil to 10 cc. 0.2*N* HNO₃ for 5 hours at room temperature, shaking every 30 minutes. It was then filtered and an aliquot evaporated to dryness. More HNO₃ was added to oxidize the organic matter, it was again evaporated and the last traces of HNO₃ eliminated by evaporation with HCl. The residue was dried on a steam bath to dehydrate SiO₂, taken up with HCl and H₂O, filtered and the phosphorus determined with ammonium molybdate by precipitating at 40°C. in a small volume and allowing the yellow precipitate to stand at the above temperature for an hour or so and finally over-night at room temperature. It was then filtered and determined volumetrically.

The total phosphorus was determined by the magnesium-nitrate method with slight modifications (1, p. 25).

The large majority of determinations of total and 0.2*N* HNO₃-soluble phosphorus were made by the writer. The remainder were made by different

⁸ The average diameter of 10 precipitates of this character was 0.64 cm.

analysts in the regular soils work. Where comparisons have been made of virgin with the corresponding cultivated soil, the work was carried on at the same time. The numbers for these are bracketed in the tables.

The soils used in the work have been divided into four general groups. While the classifications are somewhat arbitrary and not definite in all cases, they are satisfactory for the purpose. The results obtained by the short test and the comparisons with the other determinations are given in tables 2, 3, 4 and 5.

TABLE 2

Determinations on soils in which the amounts of total and easily soluble phosphorus are small

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
25002}	Cultivated	0.037	None	0.0008
25004}	Virgin	0.050	Trace	0.0015
56447}	Cultivated	0.043	None	0.0010
56449}	Virgin	0.079	Very small	0.0021
56489}	Virgin	0.031	None	0.0006
56490}	Cultivated	0.019	None	0.0006
56491}	Virgin	0.018	None	0.0006
56492}	Cultivated	0.019	None	0.0006
56493}	Virgin	0.049	Trace	0.0014
56495}	Cultivated	0.042	None	0.0008
25662}	Cultivated	0.062	None	0.0009
25663}	Virgin	0.072	Trace	0.0015
56497}	Cultivated	0.047	Trace	0.0015
56499}	Virgin	0.037	Very small, larger than 56497	0.0023
36263	Virgin	0.030	Trace	0.0009
43527	Cultivated	0.048	None	0.0004
56699	Cultivated	0.050	None	0.0011
50117	Cultivated	0.044	Trace	0.0016
56701	Cultivated	0.024	None	0.0006
56702	Cultivated	0.037	Trace	0.0014
43506	Cultivated	0.052	Trace	0.0007
56457}	Cultivated	0.030*	None	Not determined
56458}	Virgin	0.033*	None	Not determined
56485}	Virgin	0.032*	None	Not determined
56487}	Cultivated	0.021*	None	Not determined

TABLE 2—Continued

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
43526	Cultivated	0.042	Trace	0.0013
56652	Cultivated	0.027	None	0.0005
56740	Cultivated	0.031	None	0.0006
56584	Cultivated	0.022	Very small	0.0016
56741	Cultivated	0.032	None	0.0007
56586	Cultivated	0.055	Very small	0.0012
56525	Cultivated	0.018	None	0.0007
56529	Cultivated	0.020	None	0.0010
56530	Cultivated	0.026	Trace	0.0014
56531	Cultivated	0.020	Very small	0.0013
56532	Cultivated	0.030	None	0.0011
56587	Cultivated	0.041	None	0.0013
56592	Cultivated	0.064	Trace	0.0009
25424	Cultivated	0.053	Very small	0.0014
25459	Cultivated	0.021	None	0.0004
25454	Cultivated	0.024	None	0.0002
25418	Cultivated	0.031	None	0.0006
25224	Cultivated	0.053	Trace	0.0006
17902	Cultivated	0.028	None	0.0002
25020	Cultivated	0.054	None	0.0003
36453	Cultivated	0.040*	Trace	Not determined
36538	Virgin	0.074	Trace	0.0026
36539	Cultivated	0.052	None	0.0011
Average of 43 soils.		0.040		0.0010†

* Not included in average.

† That is, 2.5 per cent of the total P.

TABLE 3

Determinations on soils in which the total amount of phosphorus is small, but the amount of easily soluble phosphorus is comparatively large

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
36694	Cultivated	0.068	Very small	0.0043
36696	Virgin	0.091	Very small, more than 36694	0.0051
36792	Virgin	0.088	Trace, more than 36796	0.0028
36796	Cultivated	0.067	Trace	0.0017
56549	Cultivated	0.043	Trace	0.0028
56583	Cultivated	0.059	Moderate	0.0043
56700	Cultivated	0.053	Trace	0.0021
56526	Cultivated	0.027	Very moderate	0.0022

TABLE 3—Continued

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
56528	Cultivated	0.026	Small	0.0022
56588	Cultivated	0.039	Very small	0.0019
25227	Cultivated	0.049	Very small	0.0017
36510	Cultivated	0.070	Moderate	0.0149
36511	Cultivated	0.067	Moderate, less than 36510	0.0114
36687	Cultivated	0.087	Very moderate	0.0079
56188	Cultivated	0.080	Very moderate	0.0060
56501}	Cultivated	0.070	Moderate	0.0109
56503}	Virgin	0.074	Moderate, more than 56501	0.0125
25796}	Cultivated	0.081	Very small	0.0031
25797}	Virgin	0.092	Small	0.0074
Average of 19 soils.		0.065		0.0055*

* That is, 8.5 per cent of the total P.

TABLE 4

Determinations on soils in which the total amount of phosphorus is large but the amount of easily soluble phosphorus is comparatively small

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
*9768}	Virgin	0.142†	Very moderate, larger than 9771	0.0062†
9771}	Cultivated	0.083	Very small	0.0019
14411}	Cultivated	0.165	Trace	0.0036
Same			Trace	
*14412}	Virgin	0.199†	Moderate, much larger than 14411	0.0191†
Same}			Same	
56959}	Virgin	0.188	None	0.0024
56960}	Cultivated	0.129	None	0.0018
56742	Cultivated	0.088	None	0.0010
56527	Cultivated	0.077	None	0.0010
56585	Cultivated	0.079	Very small	0.0018
36957	Cultivated	0.111	Trace	0.0009
36775	Cultivated	0.114	Trace	0.0024
25114	Cultivated	0.157	Trace	0.0046
17287	Cultivated	0.142	Very small	0.0028

* These soils belong in table 4 and are included here for comparison with the cultivated.

† Not included in average.

TABLE 4—*Continued*

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
25910	Cultivated	0.101	None	0.0008
17285	Cultivated	0.136	Very small	0.0038
43563	Cultivated	0.112	Trace	0.0011
17480	Cultivated	0.094	Trace	0.0015
17481	Cultivated	0.127	Very small	0.0030
36798	Cultivated	0.144	Trace	0.0019
25362	Cultivated	0.103	Trace	0.0006
25228	Cultivated	0.095	Very small	0.0017
25134	Cultivated	0.107	Trace	0.0009
25135	Cultivated	0.107	None	0.0008
25995	Cultivated	0.093	None	0.0007
25311	Cultivated	0.131	None	0.0012
36688	Cultivated	0.134	Small	0.0031
5322	Cultivated	0.138	None	0.0022
56324	Cultivated	0.139	None	0.0016
56325	Cultivated	0.136	None	0.0010
43002	Cultivated	0.122	Trace	0.0014
36574	Cultivated	0.144	None	0.0010
14956	Cultivated	0.174	Trace	0.0014
2306	Cultivated	0.154	Very small	0.0023
Average of 31 soils.....		0.123		0.0018†

† That is, 1.5 per cent of the total P.

TABLE 5

Determinations on soils in which the amounts of total and easily soluble phosphorus are large

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
17483}	Cultivated	0.175	Moderate	0.0158
17485}	Virgin	0.190	Moderate, less than 17483	0.0122
2305*	Cultivated	0.204	Fair, much larger than 2306	0.0145
2306†	Cultivated	0.154†	Very small	0.0023†
56747	Virgin	0.317	Large	0.0780
Same			Same	
56784	Cultivated	0.151	Fair, much less than 56747	0.0234
Same			Same	
56733	Cultivated	0.427†	Moderate	Not determined
50592	Cultivated	0.620	Large	0.2260

* Cultivated since 1884.

† Cultivated since 1860, same farm. Belongs to table 3 but included here for comparison with 2305.

† Not included in average.

TABLE 5—*Continued*

SOIL NUMBER	CHARACTER	TOTAL P	SHORT TEST	P DETERMINED AFTER 5 HOURS' DIGESTION IN 0.2 N HNO ₃
		<i>per cent</i>		<i>per cent</i>
36790	Cultivated	0.791	Large	0.4200
25203	Cultivated	1.464	Large	0.6180
25204	Cultivated	2.227	Large	0.9600
25112	Cultivated	0.243	Moderate	0.0176
25039	Cultivated	0.205	Moderate	0.0160
50735	Cultivated	0.254	Moderate	0.0165
43407	Cultivated	0.203	Moderate	0.0171
25824	Cultivated	0.207	Moderate	0.0116
56329	Cultivated	0.148	Very moderate	0.0067
56855	Cultivated	0.107	Moderate	0.0111
25664	Cultivated	0.093	Very moderate	0.0072
25295	Cultivated	0.101	Moderate	0.0090
56188	Cultivated	0.080	Very moderate	0.0060
9768	Virgin	0.142	Very moderate	0.0062
14412	Virgin	0.199	Moderate	0.0191
Average of 21 soils.		0.387		0.1196§

§ That is, 30.9 per cent of the total P.

GENERAL DISCUSSION AND CONCLUSIONS

Assuming that the 0.2N HNO₃ method has some value, the question arises, where should the demarcation in the results come when they are to be interpreted as to the probable need of a soil for soluble phosphate. After considerable experience from a study of a large number of our soils, it is the writer's opinion that a soil should show a minimum phosphorus solubility of 0.005 per cent by this method, regardless of the total amount present. Between the range of 0.005 and 0.0075 per cent the use of phosphate may be beneficial, while above this it is probably not required.

In regard to total phosphorus, it might be stated that any soil below 0.08 or 0.10 per cent is in need of phosphate, between these figures and 0.15 per cent it may respond, and above this it is probably not necessary. However, this arbitrary classification as to the total probably depends to a large extent on its availability in the soil.

From an examination of the tables we find, therefore, that the demarcation in the results by the short test is defined by "very moderate" to "moderate." As stated above, these terms are difficult to define in some cases and in these instances may be partly interchangeable. At the time, it was the writer's judgment that the terms "moderate," "fair" or "large" indicated that no phosphate was needed, and "very moderate" that there was a doubt, and below this it was necessary. With but few exceptions it will be found that the results are in agreement with those of the 5-hour digestion. The exception may be due partly to inexperience with the tests at first, making it difficult

to define every one accurately, inasmuch as none were changed or discarded. Again, some of the variations are due to differences in the solubility of the phosphate in short and in longer periods of time, partly caused by subsequent adsorption.

The short test probably could be applied for calcium and potassium if desired, and although no attempts have been made, it is possible that by the employment of suitable apparatus and perhaps some modification in the procedure, an approximate quantitative determination could be made of phosphorus and possibly other elements which could be correlated with the figures obtained in the long digestion.

It will be observed that there are considerable differences in the behavior of soils in the weak acid digestion, and the percentages of the total phosphorus soluble in the weak acid are widely divergent. Assuming the surface $6\frac{2}{3}$ inches to weigh 2,000,000 pounds, the averages obtained in the different groups are as given in table 6.

There is no doubt that the conclusions reached by the 0.2*N* HNO₃ digestion are in accord with what we know about the soils in tables 2 and 4. Those in

TABLE 6

	TOTAL P		0.2 N HNO ₃ SOLUBLE P		PER CENT OF THE TOTAL P SOLUBLE IN 0.2 N HNO ₃
	Per cent	Pounds per acre	Per cent	Pounds per acre	
Table 2.....	0.040	800	0.0010	20	2.5
Table 3.....	0.065	1300	0.0055	110	8.5
Table 4.....	0.123	2460	0.0018	36	1.5
Table 5.....	0.387	7740	0.1196	2392	30.9

table 2 show need of phosphate and respond to phosphate treatment in the field. They represent a large majority of our soils of different types and experimental field results verify the laboratory tests. The phosphate in such soils is low, not available and is probably combined with iron and aluminum. On the contrary, the soils in table 5 represent some of our best; those which are in the blue-grass region. Many of these soils have a large content of calcium phosphate and the better class do not respond to phosphate treatment. Here again the 0.2*N* HNO₃ digestion is in accord and also the short test. The soils in tables 3 and 4 represent those regarding which any method for availability would have decided practical value. The weak acid digestion apparently discriminates between those soils in which the phosphorus exists as calcium phosphate and those where it is combined in other forms such as iron and aluminum and generally considered as not available.

The experimental field results in this state show that on those soils which need phosphate, applications of limestone and acid phosphate or rock phosphate give the best results. As the phosphate in either case is probably maintained in the soil in the form of calcium phosphate which, if true, would

more than likely be shown by the 0.2*N* HNO₃ digestion as indicated by some of the foregoing results, this shows that the weak acid digestion should have some value in practice. In fact, those who have been engaged in the chemical analysis of soils at the Kentucky station in the past, before any experimental fields were established, predicted those areas which would respond to phosphate treatment and it is with some gratification that they now find these predictions confirmed.

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THE FORMS OF NITROGEN IN SOYBEAN NODULES¹

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The forms of nitrogen present in the root nodules of legumes have been the source of much speculation. From the results of many experiments, it is known that the production of root nodules may be followed by a decided gain in the total nitrogen of the plant, but the initial form of nitrogen assimilated by bacteria is unknown. Also the nature of the nitrogenous substance or substances assimilated by the higher plant remains an unsolved problem. Perhaps the cells of the bacteria are absorbed directly by the plant, or more probably these cells are first acted upon by enzymes and thus prepared for plant metabolism. The results of chemical studies of nodules may throw some light on these problems.

REVIEW OF LITERATURE

From a review of the literature it appears that little chemical work has been done on the composition of nodules and of inoculated and uninoculated plants. Stoklasa (12) analyzed the roots, nodules and tops of soybean plants at various stages of growth and found the nodules richest in nitrogen at the blooming period. The percentage of nitrogen after the blooming period gradually decreased until at maturity the nodules contained little more nitrogen than the roots. Stoklasa (12) determined percentages of proteins, "amides" and asparagin in nodules. The protein was determined by Stutzer's reagent, the amides by nitrogen in the filtrate from Stutzer's reagent and from phosphotungstic acid and the asparagin by the ammonia distilled from magnesium oxide in the same filtrate. The results of these analyses, giving the percentage of nitrogenous compounds in dry matter of lupine nodules, follow:

STAGE OF GROWTH	PROTEIN	AMIDE	ASPARAGIN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Blossom.....	3.99	0.39	0.34
Fruit.....	1.54	0.15	Trace

¹ Part III of thesis submitted to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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This opportunity is taken to express to Professors Fred and Hart, due appreciation for their suggestions and criticisms.

Stoklasa was unable to identify ammonia in the nodules. He found a trace of nitrate nitrogen at flowering stage but this disappeared at fruiting. He showed further that when flowering plants were kept in darkness for 13 days there was an increase in asparagin but a decrease in dry matter, protein, lecithin, hexose and oxalic acid in the nodules as well as in the leaves. From these results he concludes that the constituents of the leaves and nodules were the same, and that nitrogen was obtained through the leaves and there formed "amides," and that these "amides" migrated to the nodules and there reacted with glucose to form protein.

Smith and Robinson (11) found a large increase in crude protein in inoculated as compared with uninoculated seeds, leaves and stems of soybean plants, but somewhat less nitrogen in the roots. They reported that the difference in the leaves was "true" protein, but in the stems the difference was due to "amide" nitrogen. The excess nitrogen from uninoculated roots also was due to "amides."

Whiting (16) studied the composition of inoculated tops, roots and nodules of soybeans grown in nitrogen-free sand at various stages of growth. He reported total nitrogen, water-soluble nitrogen, and nitrogen precipitated by phosphotungstic acid at various stages of growth. Sana (10) showed that bacteroidal tissue of nodules from *Vicia Faba* contained 0.935 per cent of nitrogen while the cortical layer contained only traces. The age or stage of growth when these analyses were made is not given. He identified 1-asparagin and leucin from the soluble nitrogenous constituents of nodules.

Several compounds have been suggested as the first assimilation product by nitrogen-fixing bacteria but practically no experimental data have been offered in support of any of them. The compounds have been suggested largely on the basis of the assumption of an analogy between fixation by bacteria and fixation by artificial methods. Gautier and Drouin (1) propose that nitrogen of the air is first oxidized to nitrous and then to nitric acid. Loew and Aso (6) believed that ammonium nitrite is the first compound formed. They state their belief as based on the assumption that the decomposition of ammonium nitrite into nitrogen and water is a reversible reaction. They state ammonia has not been found in nodules but that nitrites are sometimes found. Hutchinson and Miller (5) reported a considerable quantity of ammonia from nodules, but the reliability of the method employed is questionable. They determined ammonia by distilling the nodule extract from magnesia under reduced pressure. Hart and Bentley (3) showed that the amide nitrogen in asparagin is split off quantitatively by distilling from magnesia at 100°C. Since the work of Stoklasa (12) and Sana (10) as well as data presented in this paper indicate that nodules are rich in asparagin, confirmation of the presence of ammonia by some other method is necessary before drawing any definite conclusions.

Heinze (4) found hydrocarbons of the acetylene series in impure cultures of *Azotobacter* but was unable to identify them in pure culture. He believed

nitrogen first united with some compound in a manner similar to the formation of hydrocyanic acid from acetylene and nitrogen. Gerlach and Vogel (2) refuted the theory of Beijerenck and Van Deusen that a soluble nitrogen compound was first formed by *Azotobacter* by showing that soluble nitrogen was not present in cultures after fixation had taken place. Their view was similar to Heinze's. These workers called attention to the large energy requirement of bacteriological as well as artificial fixation. One thousand milligrams of glucose are required to fix 9 mgm. of nitrogen. Winogradsky (17) believed that nitrogen unites with nascent hydrogen of the cells to form ammonia.

As already stated these theories are supported by no satisfactory data. Whiting (16) pointed out that neither nitrite, nitrate, nor ammonia have ever been found in nodules or in plants grown in nitrogen-free media.

EXPERIMENTAL

Are cyanides present in nodules?

It is seen from this brief review of the literature that little is known of the forms of nitrogen in the nodule. None of the workers reported an attempt to identify the cyanide radical in nodules although it was twice suggested as the first product of assimilation. By a combination of Viehoveer, Johns and Alsberg (14, 15) methods, very small amounts of hydrocyanic acid (0.01 mgm.) may be detected. Accordingly, it was decided to attempt to identify the cyanide group by this method, using relatively large amounts of material. Preliminary tests upon both inoculated and uninoculated fresh green plants gave negative results. Test for the cyanide radical in nodules was made as follows:

About 100 gm. of nodules were collected from soybean plants containing half-grown beans. The nodules were immediately crushed between the fingers and placed in 95 per cent alcohol to check all vital activity. Only enough alcohol was used to keep the nodules well immersed. Nodules and alcohol were poured into a mortar as soon as the laboratory was reached and the nodules were further macerated. The mixture was then transferred to a distilling flask and sufficient water added to give a volume of 300 cc. Enough sulfuric acid was added to give a strength to the solution of 8 per cent acid. The flask was attached to a condenser and immersed in an oil bath at 175°C. and distilled. The distillate was collected in a solution containing 0.5 gm. of potassium hydroxide. The end of the condenser was immersed in the liquid. Two hundred cubic centimeters were thus collected. The distillate was then concentrated to 1 cc. at diminished pressure and a temperature less than 70°C. A small crystal of potassium fluoride was added and then 1 cc. of 2 per cent ferrous sulfate. The concentration was continued to dryness and 1 cc. of 30 per cent nitric acid was added. No blue color was obtained even on standing. This determination was repeated except that alcohol was first driven off on the water bath. The experiment was again repeated on nodules

of flowering plants (about 20 gm.) and on nodules from plants containing nearly full-grown seed (approximately 100 gm.). The results in all cases were negative.

Crude analyses; solubility of nitrogen

Sixty grams of air-dry nodules were collected from the "Hill Farm" of the University in addition to those used for the cyanide determinations. A crude complete analysis gave the following results:

	<i>per cent</i>
Fat	1.19
Fiber	7.49
Nitrogen-free extract	51.52
Ash	6.02

Some comparative studies were made between soybeans and nodules with special reference to the solubility of nitrogen in the latter, the results of which are given in table 1. Each solubility determination was made on the original material.

TABLE 1
Comparative analyses of soybean seeds and nodules from fertile soil

	SOYBEAN SEEDS		NODULES	
	Basis of dry matter	Basis of total N	Basis of dry matter	Basis of total N
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total N	6.20	—	4.37	—
Total N after extracted with ether	7.53	—	4.33	—
H ₂ O-soluble	+	+	1.25	28.60
N soluble in 10 per cent NaCl	4.09	54.50	1.50	34.10
N soluble in 70 per cent C ₂ H ₅ OH	+	+	0.32	7.00
N soluble in 0.3 per cent NaOH	+	+	1.58	36.00
Phosphorus	0.51	8.18*	0.26	6.50*

* Ratio P to N when N = 100.

+ not determined.

The results show that a larger percentage of the total nitrogen in soybeans is soluble in salt solution than in the case of these nodules. While the latter solution is more efficient than water as a solvent of nitrogen in nodules it is interesting to note that dilute sodium hydroxide extracts substantially the same amount as the salt solution. Nodules obtained from fruiting plants, but in a light, sandy soil poor in nitrogen, contained 5.00 per cent of nitrogen, about 40 per cent of which was soluble in water, while approximately 54 per cent was soluble in 10 per cent salt solution.

Soluble protein and protease

Osborne and Campbell (8) have shown that most of the nitrogen in soybean seed is in the form of globulin (glycinin). As shown above a large per cent of the nitrogen in nodules is soluble in sodium chloride solution. It would, therefore, be very interesting to know whether or not the globulin of nodules, if present, is identical with that found in the seed, or whether the plant is obliged to resynthesize it before storage in the seed.

An attempt was made to isolate sufficient purified protein (especially globulin) from nodules for analysis by the Van Slyke method, and thus to compare its constitution with that of glycinin, the globulin of the soybean. The method followed was essentially that given by Osborne (17). Glycinin was first isolated from the Ito San soybean. Good yields were obtained. Dr. E. B. Fred supplied 160 gm. of air-dry nodules from Ito San soybean plants grown in an infertile sand near Princeton, Wisconsin. The nodules were collected when the plants were in fruiting stage, dried in a 37°C. incubator and were then ground in a ball mill. They contained 5.00 per cent of N when air-dry.

Eighty grams of finely ground air-dry nodules were treated with 800 cc. of 10 per cent salt solution at 40°C. and squeezed through canvas. To the brown solution sufficient baryta water was added to make the solution neutral to litmus. The solution was next centrifuged repeatedly until no more suspended matter came down. The residue obtained from the first centrifuging was treated with an additional 100 cc. of 10 per cent sodium chloride. This was also centrifuged until clear.

An analysis of the two clear solutions showed they contained 2.30 gm. of N = 2.73 per cent nitrogen from nodules, or 54.6 per cent of the total nitrogen was thus obtained. The centrifuged sodium-chloride extract was then dialyzed for 4 days in cold running water. This experiment was carried out in December and January and, therefore, no bacterial action interfered. Toluol was added as a precautionary measure. No precipitate was obtained. It was found that the equivalent of 0.70 per cent nitrogen (on the basis of 80 gm. of nodules) remained. The clear brownish solution was saturated with ammonium sulfate, filtered and washed, first with alcohol, then with ether. After washing with ether the material was filtered and air-dried. This was dissolved in water and the ammonium sulfate remaining with the precipitate and then dialyzed for 4 days. Practically all the precipitate dissolved readily.

This centrifuged liquid was further tested for globulin by treating a portion with sufficient sodium chloride to give a 0.5 per cent salt solution and passing in carbon dioxide for some time. No precipitate was obtained. Apparently, the nodules contained no protein soluble in salt solution but insoluble in water. Another portion was tested by heating on the water bath at different temperatures. No change occurred until a temperature of 85°C. was attained when a precipitate appeared. Complete precipitation took place when the solution

was at a temperature of 85 to 92°C. Prolonged boiling of the filtrate from the coagulum failed to produce even a cloudiness in the solution. The bulky precipitate was washed with water, both by decantation and on the filter. It was next washed with absolute alcohol and then with ether and dried. The dark-colored precipitate weighed only 0.4 gm.

The light red, perfectly transparent, liquid was dialyzed in two times its volume of alcohol overnight, fresh alcohol was added and the liquid again dialyzed overnight. The gelatinous precipitate thus obtained was washed first with absolute alcohol, next with ether and then dried. A light brown precipitate containing 0.036 gm. of nitrogen was obtained. The small yields showed it was impossible to obtain enough protein for study. The results show that nearly all the salt-soluble and water-soluble nitrogen was non-protein in character. There was apparently no globulin, and only a small amount of albumin and proteose.

Qualitative tests on nodule extract

The neutral aqueous extract of nodules that had been freed from suspended matter by centrifuging, and from proteins by heating, was subjected to several qualitative tests with the following results:

TEST	RESULT
Biuret.....	+
Hopkins-Cole.....	-
Bromine.....	-
Xantho-proteic.....	+
Millon's.....	+
Alkaline silver nitrate.....	+
Fehling's.....	+

In view of the fact that it was impossible to secure sufficient protein for analysis it was decided to subject a hydrolyzed aqueous solution of nodules previously freed from proteins to a Van Slyke analysis and to compare these results with an analysis of glycine. It was found, however, that such a large percentage of the nitrogen in nodule extract was lost as humin that such a comparison was meaningless.

Non-protein nitrogen

Since a large amount of plants and nodules from Princeton were available, careful analyses were made of the leaves, stalks, roots, and nodules, large amounts of tissue and the same amounts of soluble nitrogen being used in all cases. The results are given in table 2. The high percentage of nitrogen precipitated by phosphotungstic acid in the nodules is notable. This was true of all the analyses made of nodules. It is interesting to note in this con-

nection that *bacteroids* which occur in the nodules of several legumes are produced outside the plant by means of certain plant bases, notably caffeine.

It is noted further that roots and leaves were both higher in basic nitrogen than the stalks. This may indicate that the basic nitrogen in the roots was influenced by the bases of the nodules. McCool and Millar showed that the sap of the roots more nearly reflected the concentration of the soil solution than did the sap of the tops. It is, therefore, possible that the composition of the nodules influences the composition of the roots more than the tops. A careful study of the basic nitrogen of the roots of plants receiving their nitrogen from nodules as compared with those receiving their nitrogen from different planes of nitrate may throw some light on this problem. Since large amounts of tissue would be necessary it would be advisable to grow the tissue in nitrogen-poor soil in the field rather than in the greenhouse. Some studies were made along this line, but the small amount of tissue available made the results inconclusive.

TABLE 2
Distribution of nitrogen inoculated soybean plants
(Expressed as per cent of water-soluble N)

	AMINO N	AMIDE N	BASIC N
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Leaves.....	27.8	17.2	27.7
Stalks.....	20.9	21.4	11.5
Roots.....	16.7	21.4	19.2
Nodules.....	16.3	19.3	62.2

Extensive comparative studies were made of the amino-nitrogen and amide-nitrogen content of inoculated and uninoculated plants. No light on the problem at hand could be obtained because the amounts of these forms present in the plant appear to depend more upon the quantity than the quality of nitrogen supplied. This was in accord with Suzuki's (13) studies on the effect of inorganic nitrogen on the accumulation of asparagin in plants.

SUMMARY

1. The first product of nitrogen assimilation by legume bacteria and the form or forms of nitrogen assimilated by the plant from the nodule are unknown.

2. Twenty to one hundred grams of soybean nodules collected in the field at the flowering and during the fruiting of the plant do not contain the cyanide radical according to a method delicate to 0.01 mgm. of hydrocyanic acid.

3. The amount and solubility of nitrogen in nodules from different varieties of soybeans obtained from different fields, fertile and infertile, and in different years, but collected at about the same stage of growth, were somewhat different. Thirty to forty per cent of the total nitrogen in nodules was soluble in water, while from 40 to 55 per cent of the nitrogen was soluble in 10 per cent salt solutions, or in dilute alkali. The solubility in the latter solvents was nearly the same.

4. A study of the kinds and amounts of soluble protein in nodules showed that they contained apparently no globulin, and only a small amount of albumin. About 3 per cent of the water-soluble nitrogen was in the form of protein and proteose.

5. Of the protein-free soluble nitrogen in the nodules about 16 per cent of the total water-soluble nitrogen was present as primary amino nitrogen and 19.3 per cent was amide nitrogen. Over 60 per cent of the total water-soluble nitrogen was precipitated by phosphotungstic acid. The amount of the latter form, based upon the percentage of total soluble nitrogen, was much larger in the nodules than in roots, tops or leaves.

6. An increase in the supply of nitrogen, either from nitrates or nodules, caused an increase in amino and amide nitrogen in the plant, but this increase was independent of the form of nitrogen supplied.

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THE CONCENTRATION OF THE SOIL SOLUTION AROUND THE SOIL PARTICLES

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INTRODUCTION

On account of the supposed adsorptive property of soils it appears to be generally believed that the solution in immediate contact with the surface of the soil particles has a greater concentration, or density, than the remainder of the solution. It is further believed that the nutritive salts in this concentrated layer are very available to plants and since the root hairs come in intimate contact with it they obtain these nutritive salts very readily.

This belief, however, of the greater concentration of the solution around the soil particles than of the mass of the soil solution has never been proved. It is deduced largely from the general principles of adsorption and from the ability of soils to take up dissolved solids.

It is the object of this paper to present experimental data and other evidences which tend to show diametrically the opposite view, namely, that the concentration of the solution around the soil particles and in the very fine capillary spaces is less concentrated than the remainder of the soil solution. These experimental data would tend also to throw some light on the question as to whether or not there really is a physical adsorption of dissolved solids by soils.

DIFFERENCE IN CONCENTRATION BETWEEN CAPILLARY-ADSORBED WATER AND FREE WATER

The evidences which go to show that the concentration of the soil solution (capillary-adsorbed water) in intimate contact with the surface of the soil particles is less than that of the mass of the soil solution (free water) may be grouped and presented under four headings: (1) The diminution of the freezing-point lowerings of soils by successive freezing and thawing; (2) the liberation of unfree water from soils by successive freezing and thawing; (3) the abnormally greater increase in the freezing-point depression of soils as the moisture content decreases; and (4) the equality in the freezing-point lowering between the supernatant liquid and the soil which it bathes.

1. *The diminution of the freezing-point lowering of soils by successive freezing and thawing*

It has been abundantly shown that the freezing-point method (3) is capable of measuring the concentration of the soil solution directly in the soil from any maximum to a very low moisture content, very readily and most accurately. It has been discovered by means of this method that the initial freezing-point lowering of soils tends to decrease with successive freezing and thawing. This is true with all soils, except the sands and the extreme coarse sandy loams, and from a very low to a comparatively high moisture content. The greatest diminution occurs, as a rule, in the second freezing, and gradually decreases in the succeeding freezings until the lowering of the freezing point becomes constant. Typical examples of these general results are shown in table 1.

TABLE 1
Effect of freezing and thawing on the freezing-point lowering of soils

SANDY LOAM			SILT LOAM		
Percentage of moisture	Number of times frozen	Lowering of the freezing point	Percentage of Moisture	Number of times frozen	Lowering of the freezing point
		°C.			°C.
3.9	1	0.430	16.4	1	0.430
	2	0.395		2	0.280
	3	0.390		3	0.220
	4	0.390		4	0.210
CLAY LOAM			CLAY		
15.6	1	0.820	18.3	1	0.870
	2	0.530		2	0.600
	3	0.520		3	0.470
	4	0.520		4	0.400

It is readily seen that the diminution of the initial depression of the freezing point decreased considerably by successive freezing and thawing. The diminution varies from 0.040°C. in the case of the sandy loam to 0.410° in the case of the clay.

The question now is what causes this diminution of the freezing-point depression with repeated freezing and thawing. Three hypotheses may be presented to explain this phenomenon:

1. The soils have the ability to cause a certain amount of water to become unfree. This unfree water may be in the soil either as capillary-adsorbed or chemically combined or both. In either event this unfree water is not free or active to function as a solvent but is removed from the active liquid phase and also from the field of action as far as the freezing-point lowering is concerned.

2. This unfree or inactive water is either entirely free of dissolved solids or contains only a small amount of them. At any rate, it is less concentrated than the solution which freezes at the first time.

3. The water which the soils cause to become unfree or inactive is due to the colloids which the soils contain and to the capillaries of the soils. A portion of this unfree water exists in the colloids as physically adsorbed and loosely chemically combined, and another portion as inactive capillary water in the capillaries of the soil. Upon freezing the colloids are coagulated, the bonds uniting them with the water break and some of the unfree water becomes liberated. The capillaries of the soil also are destroyed, or in some way affected by the process of freezing, and the inactive capillary water also becomes liberated. This liberated, free and dilute water from both sources goes to dilute the original soil solution and thereby decreases the lowering of the freezing point, just as the actual results show.

These hypotheses, therefore, would tend to explain the foregoing phenomenon, perfectly, and happily they appear to be correct, as will be seen in the next section.

2. The liberation of unfree water from soils by successive freezing and thawing

In order to prove whether or not soils do actually cause water to become unfree and thus refuse to freeze, the dilatometer method (1, 2) was resorted to. The principle of this method is based upon the fact that water expands upon freezing. Knowing the coefficient of expansion of the water upon freezing and also the total water content of the soil then it can easily be discovered whether all or part of the soil water freezes.

It was shown by this method that part of the water in the soil freezes very readily at a temperature slightly below $0^{\circ}\text{C}.$, another portion does not freeze until a temperature of $-78^{\circ}\text{C}.$ is reached and a third portion does not freeze at all. These results led to the following new classification of the soil moisture.

Gravitational	
Free	
Unfree	$\left\{ \begin{array}{l} \text{Capillary-adsorbed} \\ \text{Combined} \end{array} \right\} \left\{ \begin{array}{l} \text{water of solid solution} \\ \text{water of hydration} \end{array} \right.$

Free water is that which freezes for the first time at the temperature $-1.5^{\circ}\text{C}.$ capillary-adsorbed water is that which freezes finally between -1.5° and $-78^{\circ}\text{C}.$, minus the free water and the combined water is that which does not freeze at all.

The total amount of unfree water, or that which refuses to freeze for the first time at the temperature of -1.5° , is surprisingly large, especially in the fine-textured or colloidal soils. Thus, in Superior clay it is 22.84 per cent, in Chino silty clay loam 22.52 per cent, and in Houston clay 18.03 per cent, based on the oven-dry soil, or 84.80, 85 and 63 per cent, respectively, based on the water added, which was 5 cc. to 20 gm. of soil.

The dilatometer method also showed that the process of repeated freezing and thawing actually does liberate some of the unfree water, as more of it freezes. This fact is very convincingly shown in table 2. The soils represented in this table are exactly the same as those in table 1. The same soils were used for the data in table 2 purposely to prove that the diminution of their freezing-point lowering with succeeding freezing and thawing is due to the liberation of some of their unfree water.

It is readily seen, therefore, that the amount of water which failed to freeze in the first freezing decreased with repeated freezing and thawing, proving that some of the unfree water became free and froze. The quantity of this liberated water is considerable, amounting to 4.50 per cent in the case of the silt loam.

TABLE 2

Effect of repeated freezing and thawing on the amount of water that fails to freeze in soils

SOILS AND TREATMENT	NUMBER OF TIMES FROZEN	PROPORTION OF WATER FAILED TO FREEZE; BASED ON AIR-DRY SOIL
		<i>per cent</i>
Sandy loam 20 gm. + 1 cc.	1	1.20
	2	0.90
	3	0.90
Silt loam, 20 gm. + 3 cc.	1	10.75
	2	.00
	3	6.25
	4	6.25
Clay loam, 20 gm. + 2.5 cc.	1	8.75
	2	6.50
	3	6.25
	4	6.25
Clay, 20 gm. + 3 cc.	1	13.25
	2	10.20
	3	9.80
	4	9.80

The diminution of the freezing-point lowering of the soil, therefore, is logically explained by the liberation of some of its water which it caused to become unfree and thus removed from the active liquid phase and also from the field of action as far as the freezing-point lowering is concerned. Since the depression in this case could be diminished only by dilution, then it naturally follows that this liberated water is very dilute or less concentrated than the mass of the soil solution which freezes readily. This conclusion is irresistible and self-evident. If the liberated water had a concentration equal to that of the mass of the active solution, the depression would be the same as before. If it had a higher concentration the depression would be greater than before.

Since, however, it is less, then it logically follows that the liberated water is less concentrated than the mass of the soil solution.

The next question that should be considered is the condition in which the unfree water exists in the soil and especially of that portion which becomes liberated upon freezing and thawing.

It will be recalled that on the basis of the dilatometer method that portion of the water which freezes very readily slightly below 0° was called free water. It was considered reasonable and logical to call this water free because pure water in mass freezes at 0° . Water which does not freeze at this temperature must be different from free water. Since the physical condition of the soil presupposes that some of its water must exist around and in the interstices of its particles, and that this water probably has a lower vapor pressure corresponding to a lower freezing point, it appeared reasonable and logical to call this water capillary-adsorbed. On the other hand, water which does not freeze at all, even at the extreme low temperature of -78°C . must also be different from the capillary-adsorbed. Since it is known that certain solid materials contain water of hydration, solid solution of water, etc., it seemed reasonable to call this water combined.

This classification, therefore, not only is supported by actual experimental results but is based upon scientific principles as well.

From the above classification and discussions it is apparent that the water which becomes liberated upon freezing and thawing must be the capillary-adsorbed. It could not very well be either the free or combined because the former is already free and freezes readily the first time, while the latter probably could not become liberated if it is in the form of water of hydration or water of solid solution. Furthermore, this combined water seems to be constant in quantity in any one soil.

That the water which becomes free upon freezing and thawing is capillary-adsorbed, seems to be beyond any doubt. As will be shown in a later paper, when a moist soil freezes the water in the larger capillaries has the power to draw to itself the water from the smaller capillaries and the films from around the particles. The water in the larger capillaries, therefore, grows at the expense of the water from the smaller capillaries and of the films. Probably the greatest portion, if not all of the water which becomes liberated and freezes in the successive freezings and thawing, is due to this fact.

Of the three different forms of water the combined exists in the most intimate contact with the soil particles, then comes the capillary-adsorbed and finally the free. Even this capillary-adsorbed water, therefore, which is not in as intimate contact with the surface of the soil particles as is the combined water, has a lower concentration than the free water, or the mass of the soil solution. This would go to indicate that the radius of influence of the soil particles is probably greater than is supposed.

As will be shown in the next section the combined water also appears to be devoid of dissolved solids, or to be much less concentrated than the free water.

All the foregoing experimental results and deductions lead to one practical and inevitable conclusion, which is the main thesis of this paper, namely, the solution around the soil particles and in the very fine capillary spaces is less concentrated than the remainder of mass of the soil solution, thus contradicting the prevalent notion.

There still remain two additional sets of evidence to be presented in support of the above conclusions. This evidence forms the subject matter of the two sections following.

3. The abnormally greater increase in the freezing-point depression of soils as the moisture content decreases

If the freezing-point depression of soils is determined at various moisture contents it will be found that the depression increases at an abnormally greater rate than the moisture content decreases. Indeed the depression tends to increase in a geometrical progression as the moisture content decreases in an arithmetical progression. A typical example of these results is shown in table 3.

TABLE 3
The freezing-point lowering of a clay loam at different moisture contents

PERCENTAGE OF MOISTURE	OBSERVED LOWERING OF THE FREEZING POINT	CALCULATED LOWERING OF THE FREEZING POINT
	°C.	°C.
10.0	1.292	
12.5	0.612	0.956
15.0	0.377	0.453
17.5	0.252	0.279
20.0	0.162	0.186
22.5	0.112	0.120

If, on the other hand, the freezing-point lowering of pure coarse quartz sand is determined at various moisture contents it will be found that in this case the depression increases in an inverse ratio as the moisture content decreases. In other words, these results follow the inverse proportionality laws and are very different from those in soils. They can be expressed by the simple mathematical equation $MD = K$ where K is the resultant constant, M the percentage of moisture and D the observed depression of the freezing point. A typical example of those results is shown in table 4.

TABLE 4
Lowering of the freezing point of quartz sand at various moisture contents

PERCENTAGE OF MOISTURE	OBSERVED LOWERING OF THE FREEZING POINT	CONSTANT K
	°C.	
2	0.091	0.182
6	0.027	0.162
10	0.018	0.180
14	0.072	0.168
18	0.009	0.162

Now when water is added to a dry soil one of the first things it would do is to dissolve the salts in the soil. If the soil acted indifferently toward the water, just as the quartz sand did, the freezing-point depression would increase inversely proportionally to the water content just as in the case of the quartz sand. Again, if some of the added water, together with its aliquot part of the dissolved salts, was caused to become unfree and thus removed from the field of action as far as the freezing-point lowering is concerned, the remainder of the soil solution should give results like those of the quartz sand. Since the actual results, however, show that the concentration of the free solution increases at a greater rate than the moisture content decreases, the inference is irresistible and logical that the soil exerts a selective action, causing only the water to become unfree and leaving behind all or most of its dissolved salts.

It would appear that the validity of this conclusion could be further proven by adding to the soil a salt solution the freezing-point depression of which was known, and if the soil abstracted from the solution only the solvent, and the solute was left behind, then the freezing-point depression of the resultant solution should be greater than the original. Unquestionably such would be the result if some salt could be found which did not react with the soil but remained inactive or indifferent. However, the soil does react to some degree with all common chemical compounds and the resultant compounds formed have a different freezing-point depression from that of the solution added. On the other hand, pure quartz flour which probably does not react with salts does give the anticipated result, namely, the concentration of the salt solution becomes appreciably greater when it is added to the quartz flour. Thus, for instance, the freezing-point lowering of KCl solution is increased from 0.390° to 0.490°C. , or a difference of 0.100° , when it is added to pure washed quartz flour. This is true, however, only at very low moisture contents.

Another most interesting and significant phenomenon that should also be mentioned is the fact that the concentration of the capillary-adsorbed water in soils is less than that of the free water even when a salt solution is added to the soil and the salt reacts with the soil. It will be found that if salt solutions such as $\text{Ca}(\text{NO}_3)_2$, etc. are added to the soils and the latter are subjected to repeated freezing and thawing, results of practically the same type are obtained as in the case of pure water. Now when it is considered that the salt is not absorbed or fixed by the soil as a whole but only the base is taken up, and an equivalent amount of another base or bases is released by the soil; and when it is further considered that this fixation and exchange take place on the surface of the soil particles right where the capillary-adsorbed water is, then it seems strange and very significant that this layer of capillary-adsorbed water should be still less concentrated than the mass of the soil solution. Just what is the cause and mechanism by which this phenomenon is accomplished is difficult to say at present.

4. *The equality in the freezing-point lowering between the supernatant solution and the soil which it bathes*

If it were true that the soils had the power to condense or physically fix on the surface of their particles a layer of solution of greater density or concentration than the mass or volume of solution bathing the soil, then it would seem reasonable to expect that a soil bathed in an excess of salt solution should show a greater lowering of the freezing point than the supernatant liquid. A large number of experiments performed, however, with many different soils and various kinds of salt solutions and different periods of contact, failed to show that such is the case. Indeed the results showed in many cases that there was a difference in the depression in favor of the supernatant liquid. This difference, however, was extremely slight, probably on account of the large volume of solution used.

All the foregoing array of evidence, therefore, agrees and points overwhelmingly to the conclusion that the solution around or in immediate contact with the surface of the soil particles is less concentrated than the mass of the solution, which is diametrically opposed to the prevalent notion.

Again, the preceding results seem to make it very doubtful whether there really is a physical adsorption of dissolved mineral solids by soils.

SUMMARY

It appears generally to be believed that on account of the adsorptive power of soils the solution around or in immediate contact with the surface of the soil particles is more concentrated than the mass of the soil solution.

In this paper, however, there is presented experimental evidence which proves diametrically the opposite view, namely, the solution around the soil particles and in the very fine capillary spaces is less concentrated than the mass of the solution. All experimental evidence obtained is overwhelmingly in favor of this conclusion.

A correct knowledge concerning this particular point is of profound and far-reaching importance, for the proper understanding of both the soil solution and its utilization by the plants.

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CHEMICAL EFFECT OF SALTS ON SOILS¹

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INTRODUCTION

The solid, liquid and gaseous phases of a soil form a chemical system and a material change in any one of these phases must inevitably affect the equilibrium of the system. As is well known, there has long been a tendency among writers on soils to treat the subject as if a condition of stable equilibrium existed in soils, while as a matter of fact chemical reactions may be readily induced, and changes are continually taking place, with the consequent shifting of the equilibrium. As shown by Stewart (23), Hoagland (10) and Burd (3), the soluble components of cropped and uncropped soils, especially the former, are subject to wide fluctuations both quantitatively and qualitatively.

It is important that we extend our knowledge regarding the changes that salts produce in soils; for not until a thorough analysis is made of the fundamental equilibria involved, can we hope to have an adequate understanding of the physiological effects of alkali salts and of fertilizers in general.

Despite the many previous investigations on this subject, much still remains to be determined. It is important to have a clearer understanding of the nature of the reactions which take place when salts are added to soils, of the constituents on which they react, and of the resulting products of the reaction and their relations to the concentration and composition of the soil solution.

The fact that large amounts of soluble salts are continually being introduced into the soils of the semi-arid region, either as constituents of the irrigation water (14) or by capillary rise from the subsoil, gives special interest to a study of the chemical reactions involved.

A large part of the previous work² on this subject has been done with the use of potassium and ammonium salts (8, 22, 25, 26), such as are widely used in commercial fertilizers. When neutral solutions of these salts are brought into contact with soil, it has been shown repeatedly that for every part of potassium or ammonia absorbed by the soil, there is brought into solution a

¹ Paper No. 75, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² A very complete review of the literature bearing on the effects of salts on soils is given by Sullivan (24).

chemical equivalent, or approximately so, of other bases. Calcium is usually dissolved in greatest quantity, but appreciable amounts of magnesium and sodium also are usually set free.

While it is well known that soils have the power of fixing considerable amounts of potassium and ammonia, it is not so widely recognized that sodium salts also react with soils. In fact, it is frequently stated that sodium is not fixed by soils. There is abundant evidence, however, that sodium salts produce substantial chemical reactions with many soils. Especially is this true of the soils of the semi-arid region of America. It has also been frequently claimed that calcium salts replace potassium from soils, but various investigators have been unable to demonstrate this reaction. The obvious explanation of these discrepancies lies in the inherent differences in the soils that have been studied, as was pointed out by Lipman and Gericke (20).

The amount of the reactions taking place and the specific bases replaced by a given salt seem to depend mainly on the nature and amounts of the silicates³ present and the state of subdivision of the soil. A given base, combined in certain silicates, is far more readily replaceable than in others, Lemberg (16, 17, 18, 19) has made extensive studies on the effects of salts on minerals. His results, Eichhorn's (4) and those published more recently by Sullivan (24), are of special interest in this connection. From their investigations it was shown that salt solutions containing monovalent bases readily react with certain silicates; salts of divalent bases react with others; while neither of these classes of salts reacts with still other silicates. The reactions appear to be of the nature of an exchange of bases and are usually reversible, although the reactions do not necessarily take place with equal facility in both directions. It is probable that calcium is more easily replaced from soils than the monovalent bases.

The reaction (H-ion concentration) of salt solutions may also be materially altered by soils; and conversely, the H-ion concentration of a soil extract may be materially altered by treating the soil with soluble salts. Certain soils convert neutral salt solutions into distinctly acid solutions. Other soils, which give approximately neutral or only slightly alkaline aqueous extracts, may yield distinctly alkaline extracts after treatment with neutral salt solutions, as will be more fully discussed elsewhere.

If an adequate understanding of the effects of salts on plants grown in soil cultures is to be had, the chemical reactions induced in the soil by the treatments must certainly be considered. As suggested by Headley (7), the roots of plants must come into contact with not merely a simple aqueous

³ Many statements occur in the agricultural literature to the effect that these reactions depend on the zeolites, but so far as we are aware, the presence of true zeolites in soils has never been proven. As shown by Lemberg (18) and Sullivan (24), salts react not only with the zeolitic minerals but with various other silicates as well. For the present we prefer, therefore, to assume these reactions to be due to silicates in general.

solution of the added salt, but with a solution after equilibrium⁴ has been established between the added salt and the soil. This solution will inevitably contain the soluble products of the reactions as well as any non-reacting soluble substances present in the soil previous to the treatment. Furthermore, the evidence obtained by the determination of the freezing-point depression (2, 6), the use of the dilatometer (1) and conductivity measurements (11), indicates that the total concentration of the soil solution, to say nothing of its composition, may be materially affected as a result of adsorption, whether we consider adsorption to be a physical or a chemical process.

It follows, then, that the composition of a salt solution after having come into intimate contact with a soil, may differ materially from that of the original solution. As pointed out previously by one of us (13), various writers seem to ignore this fact, or else to consider it to be inconsequential. Especially has this been true with certain investigators of the effects of alkali salts (5).

Despite the many previous investigations, differences of view still prevail concerning the relative toxicity of the different salts and the alkali tolerance of different plants. As suggested above, the addition of like amounts of a given salt to different soils has frequently been shown to produce variable effects with a given species of plant. In many of these and other investigations on alkali soils, very little if any attention has been given to the effects of the salts on the soil itself. Various phases of this question are being studied in this and other laboratories of the California Agricultural Experiment Station.

METHODS

Two distinctly different soil types were used in this study. One soil, no. 430, was obtained from the Citrus Experiment Station, Riverside, California, and is classified as Placentia light sandy loam; the other, no. 431, from La Habra, California, is a clay loam of the Ramona series. In each case a sample consisting of several hundred pounds was taken to a depth of 1 foot. After becoming air-dry each was passed through a 2-mm. screen, thoroughly mixed and stored in bins.

Portions of each were treated with solutions of different salts in the ratio of 1 part of soil to 5 parts of solution. Four hundred grams of the air-dried soil was shaken 1 hour with 2000 cc. of the solution, then filtered through Chamberland-Pasteur tubes and the filtrate analyzed. The solutions employed were accurately standardized, being made up with the purest salts obtainable, dissolved in distilled water, from which all but traces of CO₂ had been removed by aeration.

Standard methods of analysis were employed. Usually 400 cc. of the filtrate was treated with 20 to 25 cc. of aqua regia, evaporated to dryness, the

⁴ We do not mean to give the impression that true equilibrium in the physico-chemical sense persists for any considerable period of time in soils.

residue taken up with dilute HCl and filtered to remove SiO_2 . The resulting filtrate was analyzed for Ca, Mg, K and Na. Separate aliquots were titrated with 0.05 *N* H_2SO_4 for CO_3 and HCO_3 , phenolphthalein and methyl orange being used as indicators. Chlorine was determined by titration in a separate aliquot. Nitrate was determined colorimetrically when present in small amounts and by reduction with aluminum when present in relatively large amounts. Sulfate was determined gravimetrically and phosphate volumetrically. Ammonia was determined by distillation with magnesium oxide. The pH values of the filtrates were determined colorimetrically, Clark and Lubs indicators and buffer solutions being used. The results are expressed in parts per million of the extracts.

EXTRACTS WITH WATER

In table 1 are given the analyses of water extracts of these soils. The extracts were obtained by shaking the soil with carbon dioxide-free distilled

TABLE 1
Composition of water extracts of soils
(p.p.m. of solution)

	SOIL 430	SOIL 431
Calcium (Ca).....	4	6
Magnesium (Mg).....	2	3
Potassium (K).....	3	6
Sodium (Na).....	4	4
Chlorine (Cl).....	2	4
Carbonate (CO_3).....	0	0
Bicarbonate (HCO_3).....	12	17
Sulfate (SO_4).....	4	7
Nitrate (NO_3).....	1	5
Phosphate (PO_4).....	3	1
Silica (SiO_2).....	5	5
Total soluble solids.....	48	93
pH.....	7.0	7.1

water in the ratio of 1:5. The results, as in the case of the salt solutions, are expressed in parts per million of the extracts. If desired, the data may be readily converted into parts per million of the air-dried soil by multiplying by 5.

Each of these soils had been cropped with grain for many years without irrigation or the application of fertilizer. As the data show, the solubility of each is low, especially so in the case of soil 430. It is also of interest to note that each soil gave approximately neutral extracts. Furthermore, other studies show that neither of them contains more than traces of insoluble carbonates. No. 430 is a soil of low crop-producing power, while the yields from no. 431 are still reasonably good.

EFFECT OF NEUTRAL SALT SOLUTIONS

The effects of 0.01 *N* solutions of sodium, potassium, ammonium, calcium and magnesium chlorides are shown in tables 2 and 3; the effects of the corresponding sulfates in tables 4 and 5, and the effects of sodium and potassium nitrates in table 6.

It will be noticed that the chloride, sulfate and nitrate of a given base produced approximately the same effect on a given soil, but the amount of the reactions which took place was much greater with soil 431 than with soil

TABLE 2
Effect of chlorides on soil 430
(p.p.m. of solution)

	0.01 <i>N</i> SOLUTIONS				
	NaCl	KCl	NH ₄ Cl	CaCl ₂	MgCl ₂
Calcium (Ca).....	18	41	38	175	62
Magnesium (Mg).....	6	11	9	16	83
Potassium (K).....	10	290	20	13	16
Sodium (Na).....	210	5	2	6	8
Chlorine (Cl).....	356	356	358	355	356
Ammonia (NH ₄).....			130		

TABLE 3
Effect of chlorides on soil 431
(p.p.m. of solution)

	0.01 <i>N</i> SOLUTIONS				
	NaCl	KCl	NH ₄ Cl	CaCl ₂	MgCl ₂
Calcium (Ca).....	45	84	80	153	134
Magnesium (Mg).....	12	21	19	29	45
Potassium (K).....	18	178	34	19	23
Sodium (Na).....	174	7	5	14	10
Chlorine (Cl).....	360	360	360	359	360
Ammonia (NH ₄).....			72		

430. In general, it may be said that the differences noted in these two soils are quantitative rather than qualitative. Soil 431 contains considerably more of the finer fractions than no. 430. The amount of surface exposed to the solutions was, therefore, probably considerably greater with the former than with the latter.

If we take into consideration the water-soluble constituents of these soils, it is apparent that the anion of none of the neutral solutions was materially changed by the soil. The cation content, on the other hand, was substantially modified. It appears that the reactions are largely in the nature of an exchange of bases. Simple calculation shows that for every part of base

absorbed from solution, an approximately equivalent amount of other bases passed into solution. In fact, this must be true, since neither the reaction (H-ion concentration) nor the anion content of the solutions was appreciably affected by the soil.

TABLE 4
Effect of sulfates on soil 430
(p.p.m. of solution)

	0.01 N SOLUTIONS				
	Na ₂ SO ₄	K ₂ SO ₄	(NH ₄) ₂ SO ₄	CaSO ₄	MgSO ₄
Calcium (Ca).....	18	48	40	177	62
Magnesium (Mg).....	8	12	9	15	84
Potassium (K).....	21	278	18	14	15
Sodium (Na).....	195	4	2	2	5
Sulfate (SO ₄).....	484	470	476	478	474
Ammonia (NH ₄).....			121		

TABLE 5
Effect of sulfates on soil 431
(p.p.m. of solution)

	0.01 N SOLUTIONS				
	Na ₂ SO ₄	K ₂ SO ₄	(NH ₄) ₂ SO ₄	CaSO ₄	MgSO ₄
Calcium (Ca).....	45	93	83	153	112
Magnesium (Mg).....	11	23	20	29	49
Potassium (K).....	27	183	29	21	22
Sodium (Na).....	155	6	5	4	4
Sulfate (SO ₄).....	498	483	483	474	475
Ammonia (NH ₄).....			66		

TABLE 6
Effect of nitrates on soils
(p.p.m. of solution)

	SOIL 430		SOIL 431	
	0.01 N solutions		0.01 N solutions	
	NaNO ₃	KNO ₃	NaNO ₃	KNO ₃
Calcium (Ca).....	17	44	45	89
Magnesium (Mg).....	6	11	13	23
Potassium (K).....	27	297	29	162
Sodium (Na).....	198	4	164	6
Nitrate (NO ₃).....	629	626	635	629

The amount of the reactions was least, although considerable, with sodium salts and greatest with magnesium salts. Potassium and ammonium salts produced intermediate and approximately equal effects.

Calcium is the base most readily replaced in these soils, while small increases in the solubility of potassium and magnesium also were produced by each of the salts. By comparing these data with those in table 1 it will be seen that both calcium sulfate and calcium chloride brought about increases in the solubility of the potassium and magnesium in each soil.

The effects of potassium, ammonium, calcium and magnesium salts on the solubility of sodium were variable and in no case very great. In view of the

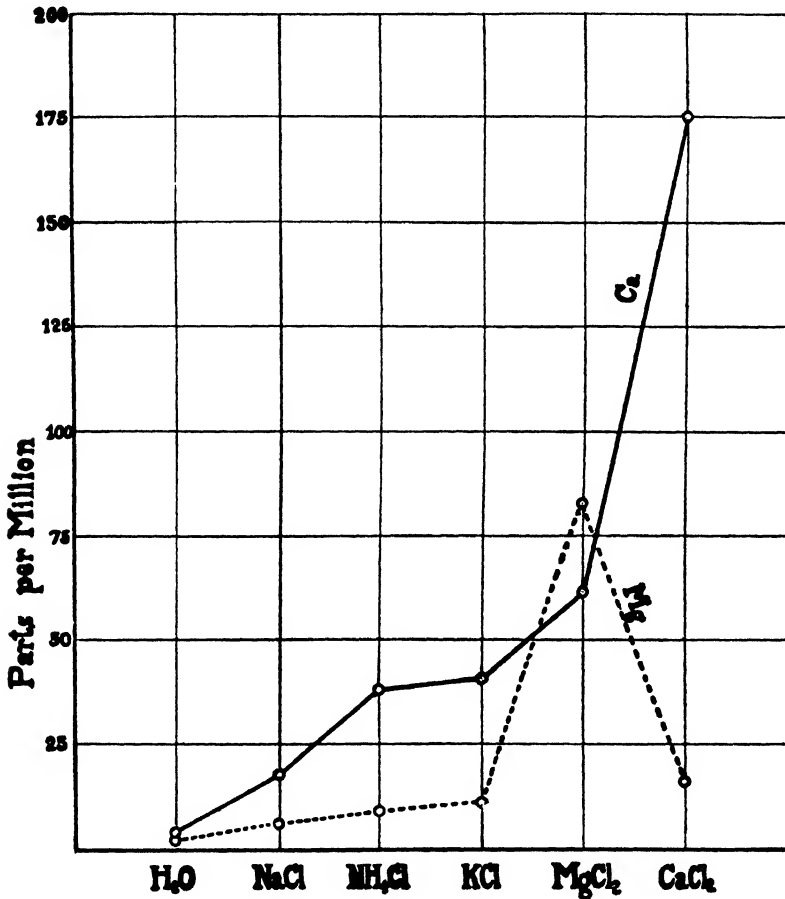


FIG. 1. GRAPHS FOR SOIL 430 SHOWING Ca AND Mg SOLUBLE IN WATER AND IN 0.01*N* SOLUTIONS OF VARIOUS CHLORIDES

magnitude of the analytical error involved in the determination of sodium, it is doubtful whether any significance should be given these variations.

The effects of the different salts are especially striking in the case of soil 431. Sodium, potassium, ammonium and magnesium salts all replaced substantial amounts of calcium from this soil. With the use of ammonium salts the extracts were found to contain greater amounts of calcium than of ammonia remaining in solution, while with the use of magnesium salts the

extracts contained more than twice as much calcium as magnesium. The results were similar, but less striking, with soil 430. The effects are well illustrated by the curves (fig. 1, 2, 3 and 4).

The H-ion concentration of most of the solutions was determined, both before and after shaking with the soil, by the use of standard buffer solutions, but as stated above, very little if any change took place.

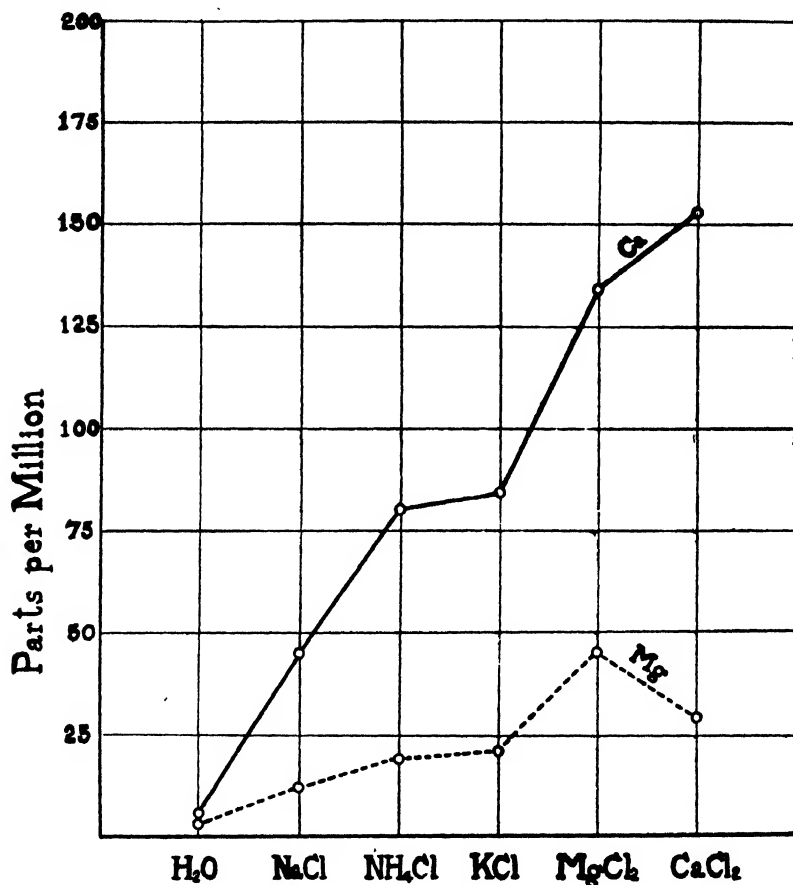


FIG. 2. GRAPHS FOR SOIL 431 SHOWING Ca AND Mg SOLUBLE IN WATER AND IN 0.01*N* SOLUTIONS OF VARIOUS CHLORIDES

These data are of interest as bearing on studies on the effects of salts on plants. In the case of soil 431 it is evident that, following the addition of any of these sodium salts, the soil moisture will contain substantial increases in the amounts of calcium in solution. As shown by Kearney and Cameron (12), Le Clerc and Breazeale (15) and others, the physiological effects of sodium chloride may be profoundly modified by the presence of calcium salts.

The data obtained by the use of magnesium salts show the extreme importance of considering the reactions induced in the soil. Kearney and

Cameron (12) have shown that while magnesium salts in simple solutions are highly toxic, the presence of soluble calcium enormously decreases the toxicity

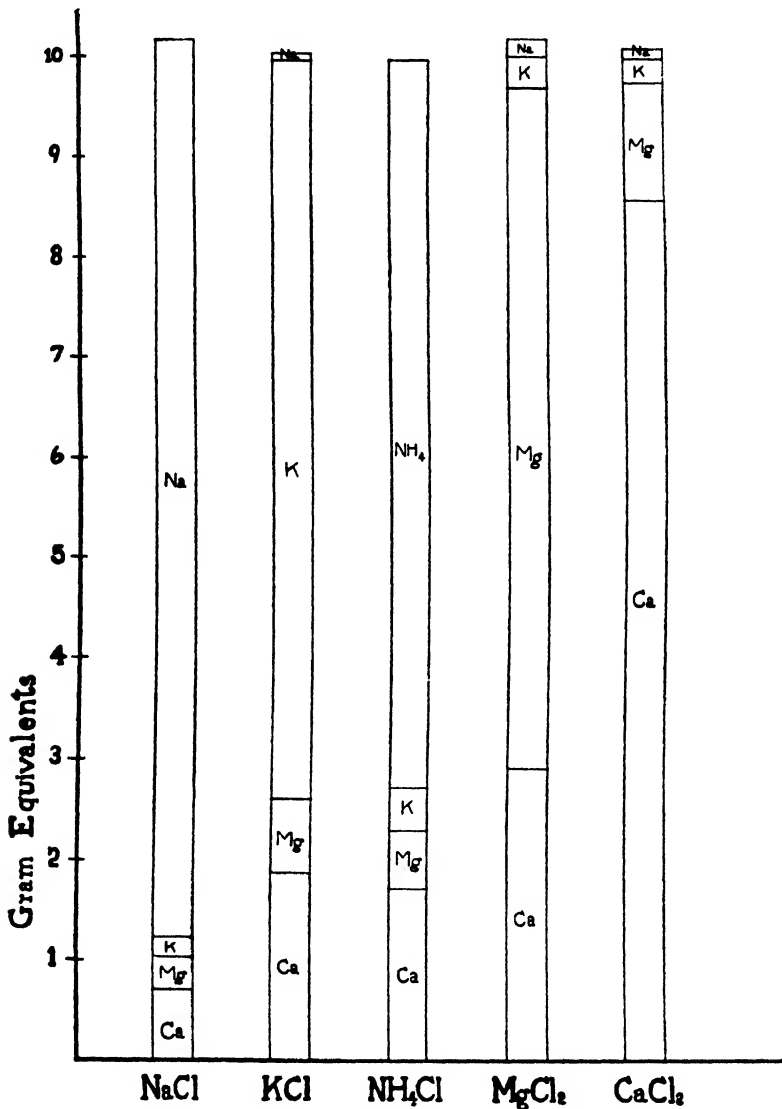


FIG. 3. GRAPHS SHOWING CATIONS FOUND IN EXTRACTS OBTAINED BY SHAKING 1 PART OF SOIL 430 WITH 5 PARTS OF 0.01*N* SOLUTIONS OF VARIOUS CHLORIDES

Calculated as gram-equivalents per 1000 liters; original solutions contained 10 gram-equivalents per 1000 liters.

of magnesium. The data show that with the use of 0.01*N* magnesium salts, plant roots in soil 431 would be in contact with a solution containing even greater molecular concentrations of calcium than of magnesium (fig. 4). In

this case, it would be more nearly correct to consider the nutrient solution as a calcium solution, than as a magnesium solution.

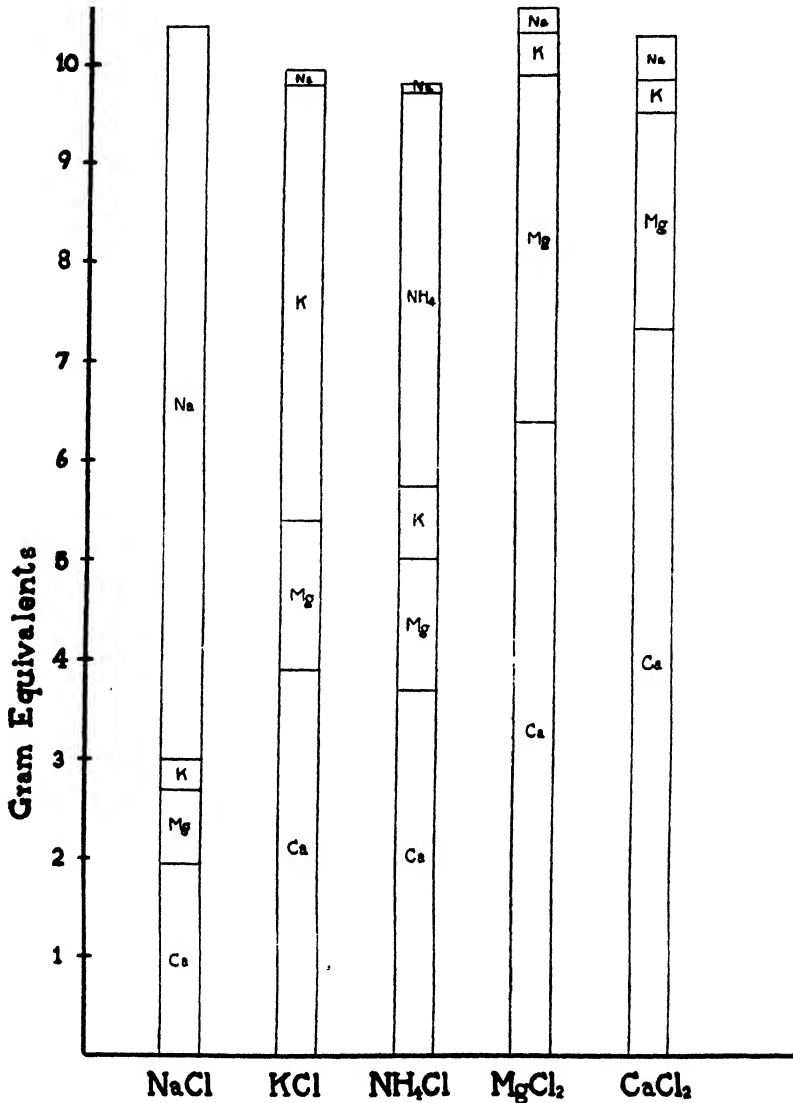


FIG. 4. GRAPHS SHOWING CATIONS FOUND IN EXTRACTS OBTAINED BY SHAKING 1 PART OF SOIL 431 WITH 5 PARTS OF 0.01N SOLUTIONS OF VARIOUS CHLORIDES

Calculated as gram-equivalents per 1000 liters; original solutions contained 10 gram-equivalents per 1000 liters.

In the light of these data it is self-evident that different physiological effects may be expected to result from the use of the same salt on different soils. Furthermore, the effects in any ordinary soil are likely to be materially different from those in sand or water cultures.

EFFECT OF PHOSPHATES

Preliminary studies have been made on the effects of soluble phosphates. The results are recorded in table 7. Solutions of di-sodium phosphate, mono-sodium phosphate and mono-calcium phosphate were employed, the strength of each solution being 0.01 *N* with respect to PO_4 . Since PO_4 is trivalent, the sodium content of the first and second of these solutions was only two-thirds and one-third, respectively, of that of the neutral sodium salts previously discussed. The solutions of mono-sodium phosphate and mono-calcium phosphate were, of course, distinctly acid. With these latter salts the chemical reactions which took place were probably of a more complex nature than with the neutral sodium salts.

It is interesting to note that in the case of soil 430 the extracts obtained by the use of di-sodium phosphate contained no more calcium than the water extract (table 1), and those obtained with mono-sodium phosphate contained very much less calcium than the extracts obtained with the neutral sodium

TABLE 7
Effect of phosphates on soils
(p.p.m. of solution)

	SOIL 430			SOIL 431		
	0.01 <i>N</i> solutions			0.01 <i>N</i> solutions		
	Na_2HPO_4	NaH_2PO_4	$\text{Ca}(\text{H}_2\text{PO}_4)_2$	Na_2HPO_4	NaH_2PO_4	$\text{Ca}(\text{H}_2\text{PO}_4)_2$
Calcium (Ca).....	4	9	54	12	17	43
Magnesium (Mg).....	4	5	6	7	7	12
Potassium (K).....	5	8	7	9	10	11
Sodium (Na).....	133	73	8	89	57	12
Phosphate (PO_4).....	304	309	291	258	275	255

salts. As will be shown later, the concentration of sodium ions in the solutions partly accounts for this difference, but precipitation of the products of the reactions as insoluble phosphates was also involved.

The original solutions contained 317 parts per million of PO_4 . After contact with the soils, the di-sodium phosphate solution contained only 304 and 258 parts per million, respectively. The soil which yielded the greater amount of divalent bases to the neutral solutions (431) precipitated the greater amount of PO_4 . The same is true with the mono-basic phosphate of sodium, although the acidity of this solution tended to prevent the precipitation of phosphate.

With the use of mono-calcium phosphate, substantial amounts of PO_4 were precipitated, probably largely on account of the action of the H ions in dissolving from the soil bases which then formed insoluble phosphates. In consequence of this reaction, the acidity of the solution was lowered and a partial precipitation of the calcium as insoluble phosphates resulted. From the

evidence at hand, we are inclined to believe that the well known power of soils to fix phosphate is very largely attributable to chemical reactions with the formation of insoluble phosphates. We believe, as in the case of other salts, that the fixation of phosphates by soils may be satisfactorily explained by giving due consideration to the solubility of the products of the reaction and to the principle of mass action.

EFFECT OF ALKALINE SOLUTIONS

In this experiment 0.01 *N* solutions of NaHCO_3 , Na_2CO_3 and NaOH , and 0.1 *N* Na_2CO_3 and NaOH were used. In addition to the bases determined previously, HCO_3 , CO_3 , OH and SiO_2 and pH values also are reported. The results are recorded in tables 8 and 9.

TABLE 8
Effect of alkaline solutions on soil 430
(p.p.m. of solution)

	0.01 <i>N</i> SOLUTIONS			0.1 <i>N</i> SOLUTIONS	
	NaHCO_3	Na_2CO_3	NaOH	Na_2CO_3	NaOH
Calcium (Ca).....	8	3	3	13	2
Magnesium (Mg).....	3	2	5	5	8
Potassium (K).....	13	13	3	7	7
Sodium (Na).....	200	167	108	2156	2045
Bicarbonate (HCO_3).....	573	287	0	830	0
Carbonate (CO_3).....	0	87	138*	2472	52†
Hydrate (OH).....	0	0	3	0	1263
Silica (SiO_2).....	3	4	19	9	54
pH.....	8.4	9.2	9.6		

* A part of the alkalinity may have been due to sodium silicate.

† Equal to 66 p.p.m. of SiO_2 .

It will be noted that the amounts of the different bases found in these extracts were widely different from those obtained by the use of neutral sodium salts. The calcium content was substantially less, particularly where sodium carbonate and sodium hydrate were used (fig. 6). The concentration of soluble magnesium also was materially less than in the neutral solutions, as was also that of potassium in the sodium-hydrate extracts. It is reasonable to infer that the same general types of exchange of bases took place with the alkaline as with the neutral salts of sodium, but that precipitation of calcium and magnesium as insoluble carbonates and silicates took place, with the result that the amounts remaining in solution were low.

It is especially interesting to note that while the sodium content of the extract obtained from a given soil by the use of sodium bicarbonate was practically equal to that obtained by the use of neutral sodium salts, the sodium was removed from solution in increasing amounts as we pass from the

less to the more highly alkaline solutions (22) (fig. 6). Each solution originally contained the same concentration of sodium. In addition to the exchange of bases, by which a portion of the sodium was removed from solution, so-

TABLE 9
Effect of alkaline solutions on soil 431
(p.p.m. of solution)

	0.01 N SOLUTIONS			0.1 N SOLUTIONS	
	NaHCO ₃	Na ₂ CO ₃	NaOH	Na ₂ CO ₃	NaOH
Calcium (Ca).....	26	7	6	23	5
Magnesium (Mg).....	7	3	5	16	13
Potassium (K).....	21	18	5	16	12
Sodium (Na).....	156	116	38	1357	1278
Bicarbonate (HCO ₃).....	531	346	46	1360	0
Carbonate (CO ₃).....	0	0	10	1152	360*
Hydrate (OH).....	0	0	0	0	785
Silica (SiO ₂).....	11	7	9	14	174
pH.....	8.0	8.2	8.5		

* Equal to 456 p.p.m. of SiO₂.

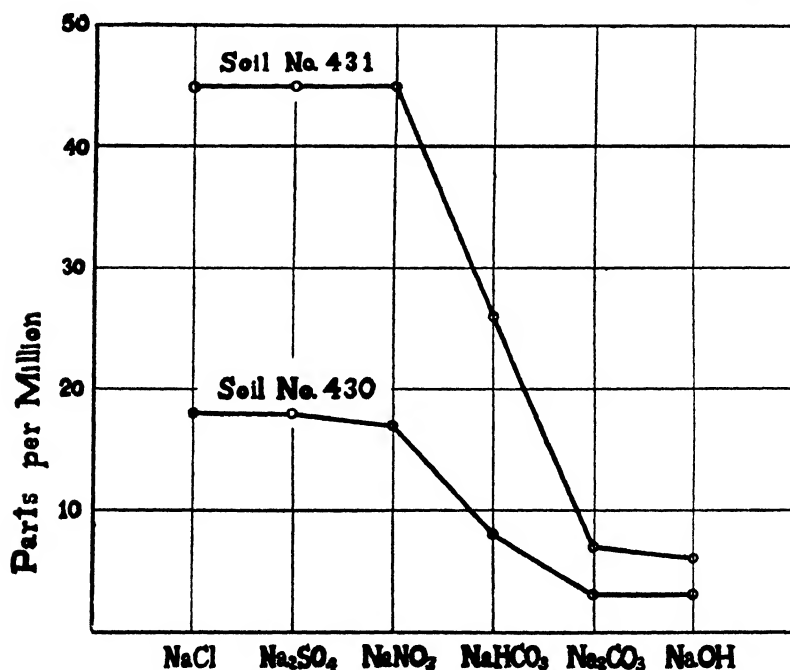


FIG. 5. GRAPHS SHOWING EFFECT OF 0.01N SOLUTIONS OF VARIOUS Na SALTS ON THE SOLUBILITY OF Ca IN SOILS

dium carbonate and sodium hydrate probably also reacted with colloidal silica with the resulting solution of small amounts of silica and the formation of colloidal sodium silicate.

The effects of the soil on the OH-ion concentration is especially interesting. The data show that each soil converted substantial amounts of normal carbonate into bicarbonate. With soil 431 not more than a trace of normal carbonate was left in the 0.01 *N* solution and more than half of the CO_3 in the 0.1 *N* Na_2CO_3 solution was converted into HCO_3 . Soil 430 produced the same type of reaction but to a much less degree.⁵ The alkalinity of the NaOH solution also was very materially lowered. In fact, soil 431 reduced the alkalinity (OH-ion concentration) of the 0.01 *N* NaOH solution to a point closely approaching that of 0.01 *N* NaHCO_3 .

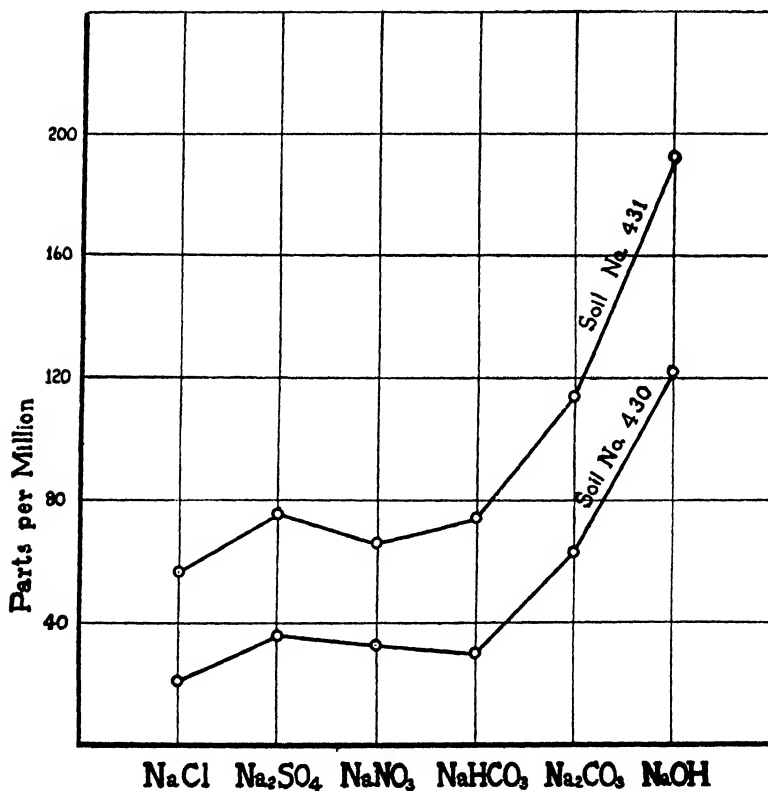


FIG. 6. GRAPHS SHOWING THE FIXATION OF Na BY SOILS (230 P.P.M. OF Na ADDED)

The effect of these soils on the OH-ion concentration of alkaline solutions is probably of a rather complex nature. Precipitation of insoluble carbonates, the solution of silica and reaction with silicic acids with the resulting evolution of CO_2 , the replacement of hydrogen from complex silicates by sodium, and reactions with organic substances, all probably played a part. The facts that only very small amounts of SiO_2 were found in the 0.01 *N* Na_2CO_3

⁵ It will be shown in a subsequent paper that the conversion of CO_3 into HCO_3 was not due to the action of CO_2 present in the compressed air used in filtering these solutions.

solutions and that the reaction of the extract from soil 431 had been reduced to a pH value of 8.2 and contained relatively large amounts of HCO_3 , suggest that the silicates of this soil may contain replaceable hydrogen. The lowering of the alkalinity, however, may have been due to reactions with organic matter. These extracts were distinctly colored and contained considerable dissolved organic matter. Whatever may be the true explanation of these data, it is apparent that substantial chemical reactions took place.

The foregoing data show at once that in studying the effects of soluble salts on plants, the chemical reactions taking place between the solution and the soil must be considered. Even with the use of a neutral salt, NaCl for example, the roots of plants will come in contact with a mixed salt solution the composition of which may vary materially in different soils.

Where the purpose is to compare the effects of neutral and alkaline salts, the reactions may become of paramount importance, and unless cognizance be taken of this fact, totally erroneous conclusions may be drawn.

The data show that the ratios of the several bases dissolved from these soils by molecularly equivalent solutions of strongly alkaline and neutral sodium salts differed widely. The anion content of the neutral solutions was not materially changed by the soils, but relatively large amounts of Na_2CO_3 were converted into NaHCO_3 with the resulting reduction in alkalinity. It is evident that no reliable conclusions can be drawn regarding the comparative toxicity of NaCl or Na_2SO_4 and Na_2CO_3 unless these reactions be definitely determined, and their physiological significance be positively established.

Since the two soils studied produce these reactions in varying degrees, and there is a strong probability that other soils will likewise vary in their power to react with salts, both quantitatively and qualitatively, it is not surprising that investigators have not been able to agree concerning the alkali tolerance of plants. It seems evident that until more complete data are obtained on the chemical reactions that take place and on the phenomenon of adsorption in soils, and more comprehensive studies are made on the physiological significance of different ionic ratios, confusion is likely to prevail among students of the alkali problem.

In view of the various reactions which take place between salts and soils and the fact that the activity of micro-organisms produces important, yet variable changes in the soluble matter of soils,⁶ it seems safe to say that water cultures must continue to be relied upon for the determination of the physiological principles underlying the effects of salts on plants. Any solid medium to be suitable for this purpose must be essentially inert towards the nutrient solution, and pure quartz sand probably affords the nearest approach to this condition.

⁶ The effects of CO_2 generated by micro-organisms in addition to that given off by the roots of plants and germinating seeds, may in itself be sufficient to lower the alkalinity of a nutrient solution very materially (9).

The above data also are of interest in connection with methods for the determination of the total alkalinity in soils containing sodium carbonate. As already suggested, sodium carbonate and other sodium salts to a lesser degree seem to react with the silicates present to form compounds relatively high in sodium, yet not readily soluble in water. Such compounds, however, are probably somewhat more soluble than the corresponding calcium salts and undergo slow hydrolysis with the continued formation, for an indefinite period, of sodium hydrate. This latter combines with the ever present carbon dioxide of soils to form sodium carbonate.

It has been found that certain soils to which sodium carbonate has been added, and the same is also true of soils in which sodium carbonate occurs naturally, continue to yield highly alkaline solutions upon repeated extraction with successive portions of pure water. In the practical reclamation of black alkali soil it is probably necessary not only to reduce the concentration by leaching away the excessive amounts of soluble salts present, but in some cases at least it may be necessary also to convert the sodium silicates into calcium silicates, as will be more fully discussed in a subsequent paper.

EFFECT OF CONCENTRATION

The solutions of the neutral salts employed above were of 0.01 *N* strength. It is also a matter of interest to study the effects of other concentrations. For this purpose seven different concentrations of NaCl were used ranging from 0.001 *N* to 0.2 *N*. The results are recorded in tables 10 and 11.

It will be noted that the more concentrated solutions replaced greater amounts of calcium, magnesium and potassium than the weaker solutions. With both soils 0.1 *N* NaCl replaced fully as much magnesium as 0.2 *N* solution, whereas the 0.04 *N* solutions replaced the maximum amounts of potassium. On the other hand, the amounts of calcium set free increased progressively with the strength of the solution. The results are well illustrated by the curves in figure 7. In general the exchange of bases follows the mass-action law.

These data are of interest from the standpoint of studies on the effects of NaCl on plants. They indicate that the composition of the soluble salts in solution in the soil moisture following the addition of NaCl of varying strengths will differ, not only quantitatively, but qualitatively as well. In other words, the roots of plants under such conditions must come in contact with solutions containing varying amounts of other ions as well as of sodium. In view of the probable influence of calcium chloride on the toxicity of sodium chloride the use of different soils in such a study introduces factors, the influence of which may materially modify the physiological effects of the NaCl and lead to erroneous conclusions regarding the toxicity of NaCl itself.

Frequently the dry salt is first mixed with the soil, then water added to effect the desired moisture content. Under such conditions it is probable

that the soil solution will contain somewhat greater amounts of calcium than if the same amount of salt were added as a solution. The reaction between soluble salts and soils is extremely rapid and since it is roughly proportional to the concentration, relatively large amounts of calcium will be set free as the particles of salt dissolve, since local zones of relatively high concentration will temporarily occur.

Preliminary studies have also been made by repeatedly extracting a given portion of soil with sodium chloride solution. By this means the products of the reaction were removed from the soil. It was found that the exchange of bases can be considerably increased over that which takes place by a single treatment.

TABLE 10
Effect of different concentrations of NaCl solutions on soil 430
(p.p.m. of solution)

	CONCENTRATION						
	0.001 N	0.002 N	0.01 N	0.02 N	0.04 N	0.1 N	0.2 N
Calcium (Ca).....	5	6	18	25	39	68	111
Magnesium (Mg).....	2	3	6	7	10	14	12
Potassium (K).....	4	5	10	7	11	8	
Sodium (Na).....	22	42	210	412	854	2171	4435
Chlorine (Cl).....	36.5	70 6	356	702	1418	3528	6957

TABLE 11
Effect of different concentrations of NaCl solutions on soil 431
(p.p.m. of solution)

	CONCENTRATION						
	0.001 N	0.002 N	0.01 N	0.02 N	0.04 N	0.1 N	0.2 N
Calcium (Ca).....	10	15	45	70	114	214	333
Magnesium (Mg).....	3	4	12	17	27	41	38
Potassium (K).....	8	9	18	14	20	18	
Sodium (Na).....	15	34	174	349	752	1951	4098
Chlorine (Cl).....	39.0	73.7	360	712	1429	3553	7021

After extracting with NaCl solution the same portions of soil were shaken with CaCl_2 solution. The results show that the sodium previously fixed by the soil is capable of substitution by soluble calcium. In other words, the reaction is reversible. It is highly probable, however, that the reverse reactions are not always easily effected. Lemberg (18) has shown, for example, that while sodium chloride may almost completely replace calcium from certain silicates, the reverse substitution in certain cases is quite difficult to accomplish, and apparently never becomes complete, though in other silicates calcium and sodium are mutually replaceable.

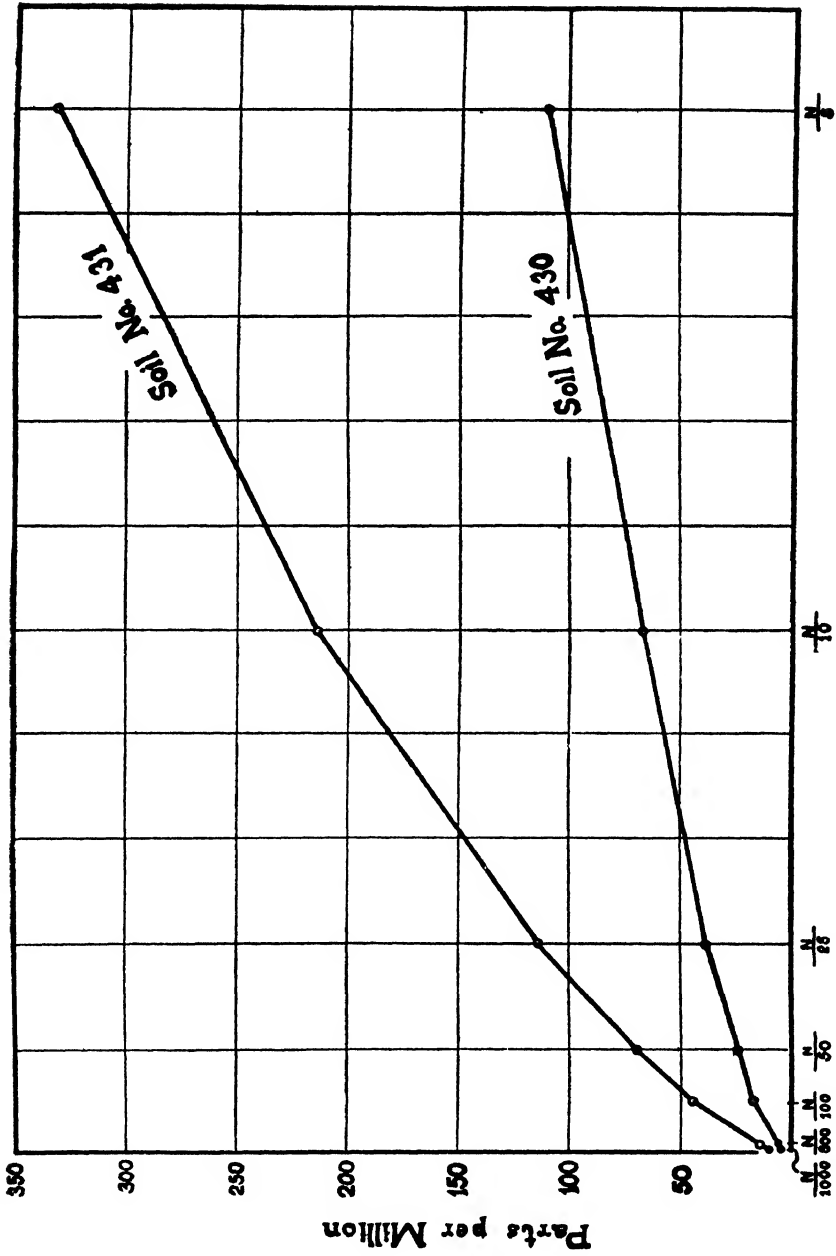


FIG. 7. GRAPHS SHOWING THE EFFECT OF CONCENTRATION OF NaCl ON THE REPLACEMENT OF Ca IN SOILS

It is reasonable to infer, therefore, that treating a soil with a salt solution not only brings about changes in the liquid components of the system represented by the soil, but also alters the chemical nature of some of the components of the solid phase as well. McCool and Millar (21) have shown, for example, that the solubility of soils after leaching in the presence of calcium sulfate is substantially greater than when leached without the addition of this salt. If a soil be subjected to prolonged reaction with sodium or magnesium salts and provision at the same time be made for removing the soluble products of the reactions, such as is afforded by leaching or the growth of crops, the reactions will continue until ultimately a greatly changed chemical system will result. Brief treatment, of course, will produce less pronounced changes; yet we must conclude that only very limited treatment with a soluble salt inevitably leaves its imprint on soils. It is probable that no soil is composed of exactly the same chemical individuals subsequent to treatment with salts, as it did previous to treatment. The physiological significance of these facts will be discussed elsewhere.

SUMMARY

1. Chemically equivalent solutions of the chlorides, sulfates and nitrates of a given base, produced substantially equivalent chemical reactions in the soils studied.

2. The solubility of the anion of the neutral salt solutions was not materially affected by the soils studied, but an exchange of bases took place, with the result that a portion of the base of the added salt passed out of solution and a chemically equivalent amount of other bases was set free from the soil silicates.

3. In the extent to which the simple salts produce these reactions with the soils studied they stand in the following ascending order: calcium, sodium, ammonium, potassium and magnesium.

4. Calcium is the base most readily replaced from these soils, but the solubility of magnesium and potassium also was increased to some extent. It is not certain that significant amounts of sodium were set free by any of the salts used.

5. Considerable amounts of PO_4 were precipitated by each of these soils.

6. Chemical reactions take place between soils and alkaline solutions which result in the conversion of normal carbonate into bicarbonate, a material lowering of the OH -ion concentration of the solution and the precipitation of greater amounts of the cation of the solution than takes place with neutral solutions.

7. The reactions between neutral salts and soils are dependent on the concentration and apparently obey the principle of mass action. Evidence has been obtained that the reactions are reversible.

8. It has been pointed out that the solution remaining in contact with the roots of plants following the addition of a soluble salt to soils must necessarily be different from that of a simple aqueous solution of the added salt. Bases are brought into solution from the soil silicates the amounts of which will vary in different soils, and the OH-ion concentration of alkaline solutions may be very materially lowered. Unless these facts are recognized, totally erroneous conclusions may be drawn concerning the relative toxicity of different salts and the alkali tolerance of different plants.

9. It is suggested that the continued addition of soluble salts in the open field where the products of the reactions are removed by either the growth of crops or intermittent leaching must ultimately result in building up a chemical system different from that originally present. As will be shown later, the physical properties of the system also may be materially altered.

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MINNESOTA GLACIAL SOIL STUDIES: I. A COMPARISON OF SOILS ON THE LATE WISCONSIN AND IOWAN DRIFTS¹

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INTRODUCTION

About 99 per cent of the surface of Minnesota was traversed by one or more ice sheets during the glacial period and practically all its soils have been formed upon the resulting mantle of glacial drift, and the aeolian and lacustral deposits derived from this. Three of the ice invasions, coming from the northeast, left reddish-colored deposits, while the others, from the northwest, left gray drift. Where the soils have been developed directly upon the glacial mantle the question arises as to what relation, if any, exists between their composition and the age and source of the drift upon which they are found.

The drift from the earliest invasion from the northwest—the Nebraskan—appears to have been everywhere buried or obliterated by the later glaciations. The latest, known in Minnesota as the Young Gray Drift, covers the greater portion of the state, and is flanked on the southeast and southwest by a margin of Old Gray Drift intermediate in age. The ice sheets that deposited these radiated from centers northwest of Winnipeg and in their advance to the southward brought a great quantity of calcareous debris, consisting of boulders, cobblestones, gravel, and rock-flour derived from the Paleozoic limestones west and south of Lake Winnipeg. As the composition of the till of these drifts when first laid down appears to have been very similar, any marked differences found in the character of the soils formed upon them may be associated with differences in their relative ages.

In such an investigation it does not suffice simply that the soils chosen for study should have developed upon drift sheets originally similar in character while differing greatly in age. It is highly important that they should have had similar surface drainage conditions, have been of similar texture, have been exposed to a similar climate and have borne a similar vegetation. Lastly, they should be as nearly virgin as possible. In Rice County (fig. 1), in which Young Gray Drift occupies the west side and Old Gray Drift the east, these conditions could be complied with. On both sides of the dividing line the rolling lands were covered by deciduous forest and the more level areas by prairie.

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SOIL AND SOIL-FORMING AGENCIES

The depth of the superficial layer that is included under the general designation "soil" varies from writer to writer, some considering it to extend only as deep as plant roots penetrate while others insist on including the full depth to which the parent rock has been affected by percolating water. In southeastern Minnesota the crops derive their mineral nutrients and their water supply chiefly from the first 3 feet of soil, and with the possible exception of alfalfa, almost exclusively from within the first 5 feet. Hence, while in agronomic questions pertaining only to that part of the state we scarcely need consider the portion below the fifth foot, the whole of the first 3 feet is to be regarded as well within the root zone.

The surface of a till plain or a terminal moraine, that has just been exposed by the recession of an ice sheet, is to be regarded not as *soil*, but as the parent rock upon which soils may be developed through the agency of percolating waters, carrying carbon dioxide and soluble salts, combined with that of living organisms and the organic compounds formed upon their death. The form of alteration induced in the parent rock by these agencies will depend upon the vegetative covering, temperature, precipitation, rate of evaporation, and level of the ground-water, as well as upon both the perviousness of the parent rock to descending water and the water-retaining capacity of its superficial portion. The character of the vegetative covering, in its turn, will be determined in part by the climate and in part by the character of the parent rock, or of the soil developed upon this. Accordingly, the character of a soil will depend upon both the properties of its parent rock and the climate where it has been formed. Older soils generally are more dependent upon the climate than upon the parent rock. Thus a granite, a wind-laid silt loam, and a lacustral clay may weather to produce soils of very similar character, when all three have been exposed for a sufficient length of time to the same climate, while they will give rise to soils of entirely different character under other climatic conditions.

DRIFT SHEETS EXPOSED IN MINNESOTA

Ten or twelve successive stages are generally recognized in the Pleistocene, or Glacial Period, in North America. Some of the ice sheets radiated from Keewatin centers north or northwest of Minnesota, and others from Labradorian centers east of James Bay. Each invasion, with the possible exception of the last, appears to have been separated from its immediate predecessor by an interglacial interval during which a milder climate prevailed, drainage channels were formed, vegetation flourished on the freshly exposed drift and soils were developed. Each succeeding ice sheet planed off most of the surface over which it advanced, cutting down the elevations, filling most of the valleys, and burying the earlier deposits under a fresh mantle of glacial debris. Drift from the earlier glaciations was more or less incorporated into

the mass of fresh material carried by the advancing ice. On most of the traversed areas the soil was thus removed but here and there tracts escaping the general abrasion were buried by the new drift. When now exposed in cuts the old soils on these furnish part of the evidence of interglacial intervals.

The generally accepted classification of the Pleistocene (1, p. 56; 6, p. 383) in the interior of North America is as follows:

- 6a. Recent epoch—Glacio-lacustrine and Champlain substages.
6. Later Wisconsin, the sixth advance. In Minnesota includes the Young Gray Drift, the Young Red Drift of the Superior Lobe and the Patrician Readvance.
- 5a. Fifth interglacial interval, as yet unnamed. The interval may have been too brief to permit of leaching and soil formation.
5. Earlier Wisconsin, the fifth invasion. In Minnesota the Young Red Drift or Patrician.
- 4a. Fourth interglacial interval—Peorian.
4. Iowan, the fourth invasion. Glacial geologists are not entirely agreed as to the separate identity of this, and in Minnesota the Iowan has been mapped along with the Kansan as Old Gray Drift.
- 3a. Third interglacial interval—Sangamon.
3. Illinoian, the third invasion. In Minnesota represented by a small area of Old Red Drift.
- 2a. Second interglacial interval—Buchanan or Yarmouth.
2. Kansan, the second invasion. In Minnesota known as the Old Gray Drift and exposed in southeastern and southwestern counties.
- 1a. First known interglacial interval—Aftonian.
1. Nebraskan, Pre-Kansan, or Jerseyan, the first invasion.

The earlier stages of glaciation appear to have been much longer than the later and some of the interglacial intervals probably much exceeded the time which has elapsed since the disappearance of the last ice sheet.

All the above-mentioned glacial invasions crossed what are now the borders of Minnesota. The first, or Nebraskan, radiating from the Keewatin field, covered the greater part of the state, extending far beyond its southern boundary. The drift from this has been completely mantled by later deposits and no natural exposure is known in Minnesota, it being encountered only in deep wells and in an occasional railroad cut or excavation.

The second, or Kansan, also from a Keewatin center, appears to have covered every part of the state except the extreme southeastern corner—the Minnesota portion of the Driftless Area, which is more extensively represented in the neighboring portions of Wisconsin and Iowa. Part of the mantle left by this ice sheet is exposed as Old Gray Drift in two southwestern and in several southeastern counties, a narrow belt lying between loess-covered Kansan on the one side and Late Wisconsin or Iowan on the other.

The third, or Illinoian, coming from the northeast, barely entered the state to the east and southeast of St. Paul, leaving the deposit known as the Old Red Drift.

The fourth, or Iowan, has been a subject of controversy since it was first described by the late Dr. Calvin of the Iowa Geological Survey. The divergent views are indicated in the next section.

The next invasion, the Early or Earlier Wisconsin, from the northeast, covered a considerable area to the west and southwest of Lake Superior (fig. 1), leaving the Young Red Drift, which extends to some little distance south

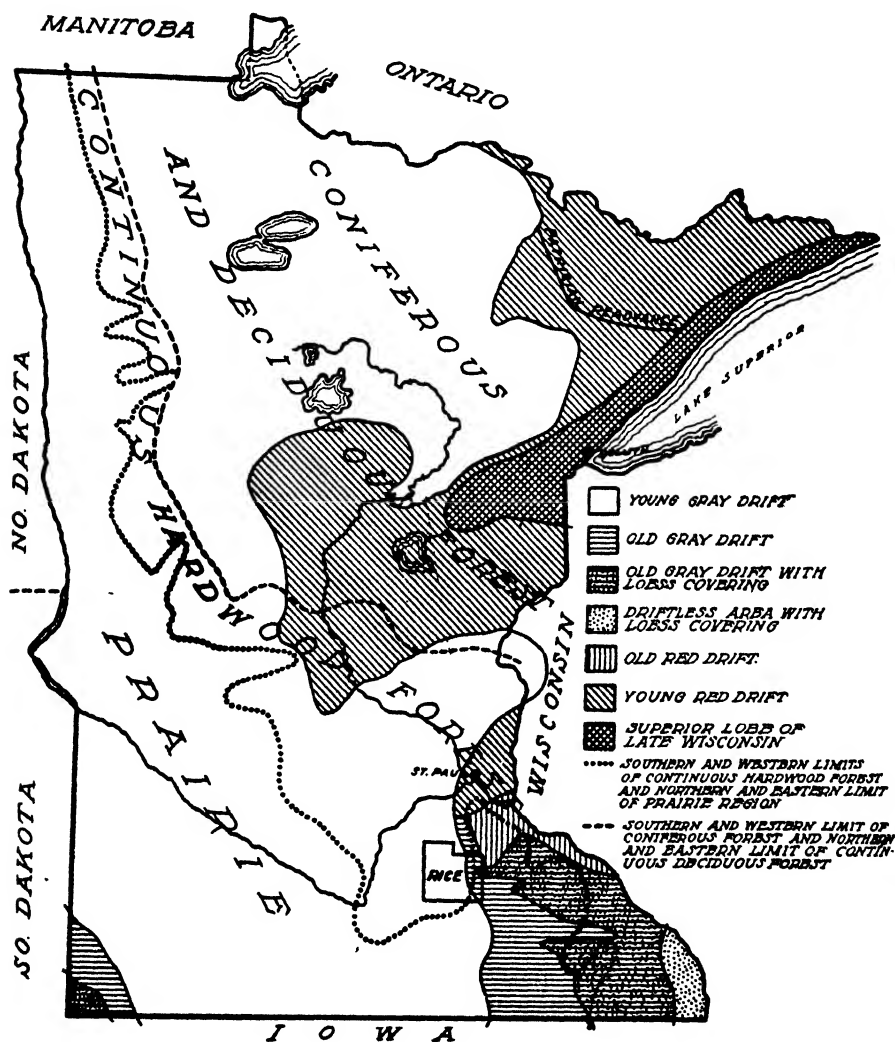


FIG. 1. MAP OF MINNESOTA SHOWING LOCATION OF THE AREA STUDIED (RICE COUNTY), AND ITS RELATION TO THE DIFFERENT GLACIATIONS AND THE NATURAL VEGETATION

After glacial map of Leverett and Sardeson (11) and vegetation map of Rosendahl and Butters (13, p. 111).

of St. Paul, even into the northern edge of Rice County. Part of the drift from this was covered by that of the next glaciation.

In the last invasion, the Late Wisconsin, there appear to have been three simultaneous advances of the ice, each from a different center. The most

extensive radiated from a Keewatin center to the northwest of Minnesota, covering a much larger portion of the state than any of its predecessors following the Kansan. The mantle left by it, and known as the Young Gray Drift, overlies most of the older drift from the northwest. In contrast with the Old Gray Drift the younger formation is characterized by numerous lakes, marshes and swamps. Fully developed drainage lines are found only where these were formed by the torrential streams which prevailed as the front of the last ice sheet was receding. The main body of the ice advanced far into Iowa and its deposit is referred to as the Des Moines Lobe (fig. 2), while an offshoot, spreading northeastward from north of St. Paul to a little beyond the

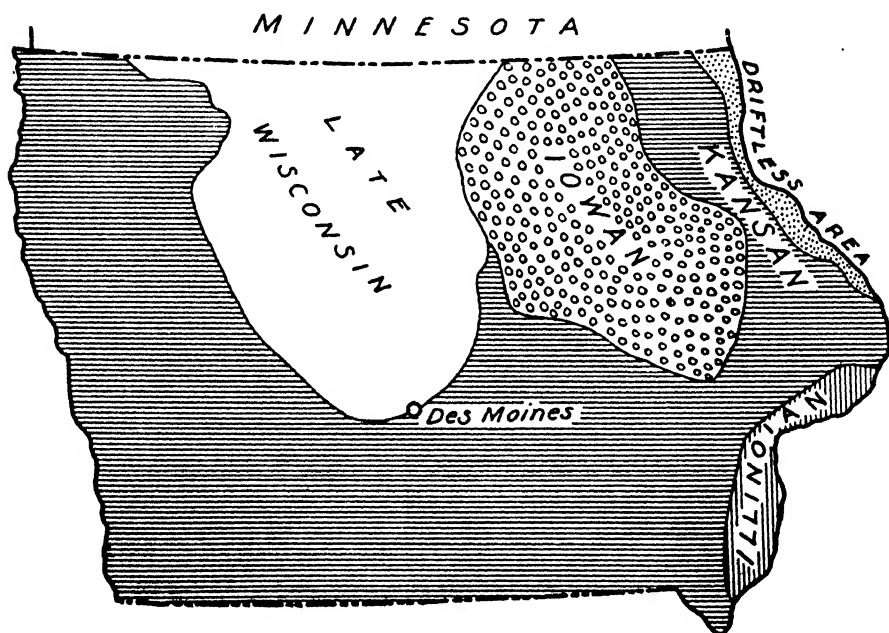


FIG. 2. GLACIAL MAP OF IOWA

After Alden and Leighton (1)

Wisconsin line, is known as the Grantsburg Lobe. In addition to these two lobes, which together constitute the Young Gray Drift of Minnesota, there was a contemporaneous Superior lobe, in which the ice advanced southwestward along the bed of Lake Superior, spread out to the north, west and southwest and left a red till. At the same time there appears to have been a readvance of ice from the north, and Leverett refers to this, in a private communication, as the Patrician Readvance.

The older drifts have been exposed to a much longer leaching than their place in the above tabulation might suggest. Using as a time unit the period that has elapsed since the Late Wisconsin drift was first exposed to erosion, and "a collation of the judgment of five of the glacial geologists who have most

studied the data in their most favorable expressions," Chamberlin and Salisbury (6, p. 413-414) report the following estimates of the relative ages:

	<i>time units</i>
From the Late Wisconsin to the present.	1
From the Early Wisconsin to the present.	2 to 2½
From the Iowan to the present.	3 to 5
From the Illinoian to the present.	7 to 9
From the Kansan to the present.	15 to 17
From the Nebraskan to the present.	x

The Nebraskan, although clearly much older than the Kansan, is everywhere buried by later deposits, thus preventing the application of estimates based upon erosion.

ADVANTAGES OFFERED BY RICE COUNTY

For such a study as that here reported Rice County appeared to offer a better opportunity than any other area in the state. The youngest drift occupies part of it while the rest is covered by an older gray drift (fig. 3). Both were deposited by ice sheets radiating from the same general center and bringing similar calcareous and clayey material from the northwest. Lying side by side they have been exposed to the same climatic influences following the last recession of the ice. Prairie as well as forest occurs naturally on both. On land with similar topography, and with the same vegetative covering, we were able to find soils similar in texture. A long period of time, including at least one interglacial period, in which floras and soils had been developed, had elapsed between the first growth of vegetation on the older drift and the exposure of the younger.

We had the advantage of a soil survey of the county, made in 1909 by the United States Bureau of Soils (4), and before we selected our fields for sampling in the fall of 1914 Mr. Frank Leverett, of the United States Geological Survey, who was just completing a detailed study of the glacial history of the state, kindly accompanied one of us over the full length of the dividing line between the two drifts in the county.

At the time the soil survey was made the existence of two drifts in the county was recognized (4, p. 21-23), Leverett having personally indicated the boundary to the surveyors, but in the report no attempt was made to differentiate soil types on the basis of difference in age of the drift, although it is stated that "the limestone from which much of this drift (Kansan) is derived, has long since given way to the agencies of weathering and only the more resistant rocks are left, whereas in the Wisconsin drift limestone and shales in addition to numerous cherty and crystalline rocks are very common" (4, p. 21).

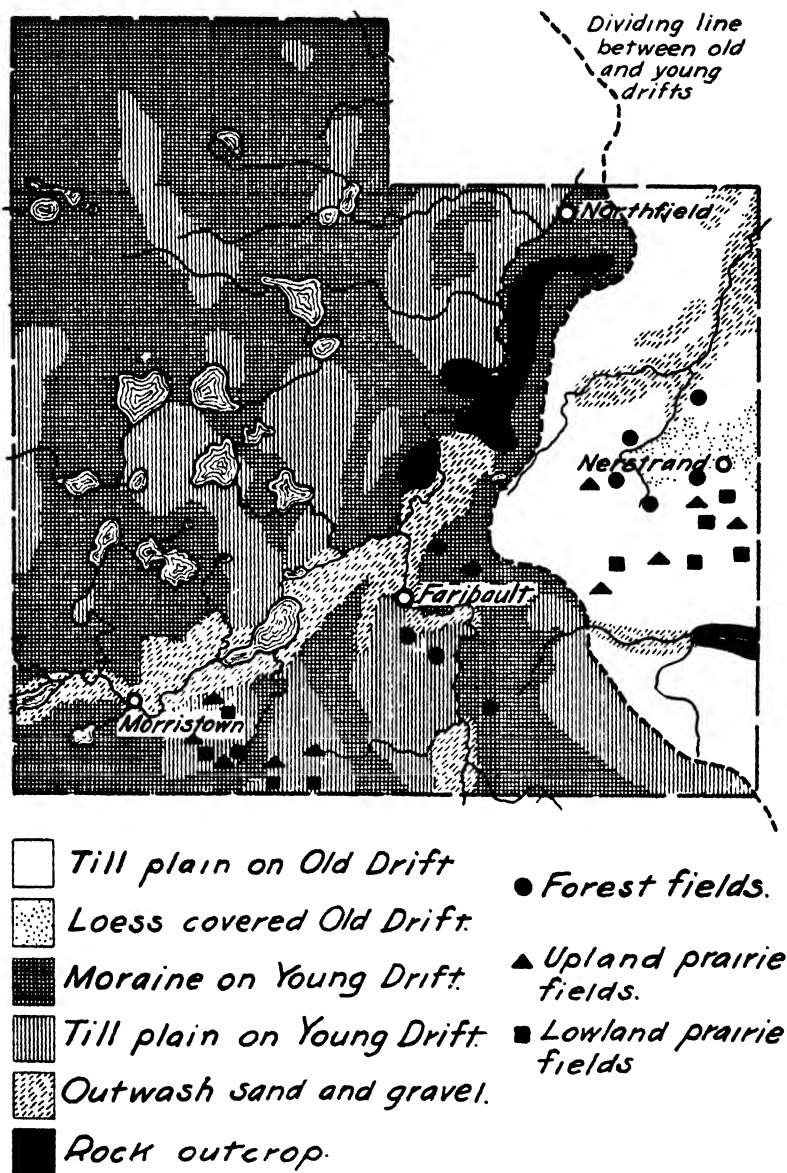


FIG. 3. MAP OF RICE COUNTY, SHOWING SURFACE FORMATIONS (AFTER LEVERETT AND SARDESON (11)) AND THE LOCATION OF THE THIRTY FIELDS SAMPLED

AGE OF THE TWO DRIFTS IN RICE COUNTY

Glacial geologists are agreed as to the general outlines of the eastern side of the Des Moines lobe, and hence as to the age of the younger drift in Rice County, and also as to the drift which extends eastward from the Des Moines lobe to the Driftless Area being much older, but they are not agreed upon the

age of the latter, as to whether it is Kansan, Illinoian, or Iowan, or partly one and partly another. Some consider it as of post-Illinoian but pre-Wisconsin age, and hence as Iowan, while others are not satisfied that it may not be either Kansan or Illinoian. On a recent map of the Iowa Geological Survey (1, pl. 14) the older drift in northeastern Iowa is shown as chiefly Iowan, with a fringe of Kansan, while an equally recent map of the Minnesota Geological Survey (11) indicates only one older drift exposed in southeastern Minnesota (fig. 1 and 2).

The question of the age of the older drift, and hence its identification, is very important. If it is Kansan with the surface exposed since Kansan time, and its soils are similar in composition to those on the nearby Late Wisconsin, it would appear useless to seek for characteristic differences by comparisons of soils on any other two drifts originally similar in composition, unless they had been exposed to climatic conditions more conducive to leaching than those which have prevailed in Rice County. On the other hand, the lack of evidence of leaching in the case of Iowan compared with Late Wisconsin, does not exclude a probability of a distinct difference between Late Wisconsin and Kansan, or even between Iowan and Kansan. This is well illustrated by Kay's explanation of the formation of "gumbotil" on the older drifts—Nebraskan, Kansan and Illinoian—as a product of long weathering and leaching, and its absence from the Iowan and Wisconsin as due to the relative youth of these (9).

About thirty years ago W. J. McGee showed that in northeastern Iowa, the part east of the Wisconsin moraine, there are two drift sheets differing greatly in age (12, p. 472 and p. 496). Later Calvin differentiated three distinct drift sheets as the Nebraskan, Kansan and Iowan. The existence of the last as distinct from the Kansan and Illinoian was questioned in a paper by Leverett (10, p. 280-283), to which Calvin replied with a summary of the evidence (5). Very recently the whole question has been reviewed by Alden and Leighton (1), who spent the summers of 1914 and 1915 on field investigations in northeastern Iowa. They conclude that there is a post-Kansan drift sheet older than the Illinoian and younger than the Wisconsin, and that there is warrant for the continued use of the Iowan as one of the major subdivisions of the Pleistocene classification. The drift of the Kansan and Iowan is so similar lithologically that the boundary line between the two has had to be determined largely upon the basis of topography—where the smooth swale topography gives way to sharply dissected topography.

In the Iowan area, though it is not generally dissected by sharp-cut, eroded valleys, there are yet present nearly everywhere the main features of maturely branching stream-erosion systems. The valleys divide and subdivide in dendritic fashion, and their branches reach most parts of the area. . . . In the Kansan there is not only deep dissection but the ramifications of the branches are developed in minute detail down to ravines and gullies trenching the slopes at intervals of a few rods. Convex curves prevail on the slopes and more or less sharply cut, V-shaped, cross-profiles predominate except in the broader flat-bottomed valleys. . . . In the Iowan drift area . . . V-shaped cross-profiles are rarely seen. The side slopes are long and of low grade. . . . The minor valleys are thus broad open swales (1, p. 61-62).

In the Iowan they find a lesser depth of leaching of carbonate, only from 4 to 6 feet in general, measuring from the surface, compared with 12 to 15 on the Kansan, while the till also is oxidized to a lesser depth, 7 to 15 on the Iowan compared with 15 to 25 on the Kansan. The characteristic *ferretto* of the Kansan, a reddish brown or dark brown zone, 1 to 1.5 feet thick and consisting of a dense sticky clay, is absent from the Iowan. On the Kansan they found gumbotil covered by loess, by Iowan drift, or by both, but on the Iowan gumbotil is absent.

Leverett and Sardeson, referring to the southeastern part of Minnesota, and with Alden and Leighton's review of the Iowan question in mind, recognize an area in which the general depth of leaching is less and the surface not so deeply reddened by oxidation, and in which the valleys are in some cases mere swales and shallower than in the Kansan drift area in southern Iowan, but "because of the vagueness of the features the limits of the Iowan drift, or the extent of Iowan glaciation, are matters on which there is a wide difference of opinion. . . . There has been perhaps a slight removal of the leached and weathered surface of the Kansan drift in exposed locations and redeposition of this material in the valleys and depressions. The valleys of this problematical area often head in shallow draws or sloughs which are wet and ill drained, but the district is entirely free from lakes and inclosed basins such as abound in the later or Wisconsin drift region" (11, p. 49). As yet no attempt has been made to trace the eastern boundary of the Minnesota portion of this problematical area.

Our forest and upland prairie fields east of the boundary line have the mantled mature-erosion type of topography which Alden and Leighton consider characteristic of the Iowan, show no *ferretto*, and the depth to which leaching of carbonate has penetrated averages only between 4 and 5 feet from the surface. As the older drift where our samples were collected thus appears clearly to belong to what these authors describe as the Iowan, we may treat it as such without reference to the question of the retention of the Iowan as one of the major subdivisions of the Glacial Period.

SOIL TYPES

Fourteen soil types have been mapped in Rice County, but only three of the most extensive have representative areas on both sides of the boundary between the drifts, viz., (1) a forest-covered type (Carrington loam); (2) an upland prairie (Carrington silt loam); (3) a lowland prairie type (Fargo silt loam). These three were selected for our study.

The Carrington soils are derived from the weathering of the glacial drift. Carrington loam occupies the rolling uplands, the moraines in the case of the younger drift, is generally well drained and originally was nearly all covered with deciduous forest—part of what is known as the Big Woods. Winchell in his survey of the county made over 40 years ago (18, p. 652-654), mentions more than thirty species of trees, the most common ten, arranged in order of

frequency, being the following: basswood (*Tilia Americana*), white elm (*Ulmus Americana*), black oak (*Quercus velutina*), bur oak (*Quercus macrocarpa*), silver maple (*Acer saccharinum*), aspen (*Populus tremuloides*), sugar maple (*Acer saccharum*), slippery elm (*Ulmus fulva*), black ash (*Fraxinus nigra*), butternut (*Juglans cinerea*). The silt loam is found on the till plain and is confined to those higher lying portions of the county which were originally covered by prairie. Boulders, some very large, occur on both types. The Fargo slit loam, originally grassland, is a bottom-land type with a topography generally level, or with a very gentle slope toward a stream or bog, usually poorly drained and often with the water-table within 3 feet of the surface.

COLLECTION OF SAMPLES

Selection of fields

We collected samples from tracts that were still in as nearly virgin condition as it was possible to obtain. At the time the samples were collected, the fall of 1914, a few of the original settlers were still living and these were able to give much valuable information. Each site was selected only after the oldest settlers in the neighborhood had been consulted and a more or less complete history of it had been obtained. As an illustration of the value of such inquiries we might mention the case of an apparently natural grassland field occurring on silt loam soil. Living within a half-mile of the field was a very old gentleman who 56 years before had cleared the brush from it and every year since had seen the growth upon it.

The locations of the 30 sites finally selected are shown in figure 3, to all of which we will refer as "fields" although in the case of the upland prairie we did not find a single entire field that had never been plowed. In the case of this type, the most desirable of the three, the selection of satisfactory sites was a difficult task and finally all of the samples representing it were taken from along fence lines where the original sod had never been plowed so far as the oldest living settlers knew. In the case of the lowland prairie virgin fields were almost as difficult to find. On the Old Drift two rather poorly drained meadows provided two of the sites and both of these fields were plowed a few days after the samples were taken. Grassy roadsides were chosen for two others, and a line fence border for the fifth, while on the Young Drift all were from line fence borders or roadsides. The required number of satisfactory and properly distributed fields on the forest type were comparatively easy to locate. As both prairie types are so easily brought under the plow it is not surprising that fields in a virgin condition on them are now so rare.

In the case of each type on both drifts an effort was made to locate the five fields so that no two would be less than a mile apart and usually this was found possible. The general appearance of the fields on the three types is illustrated by plates 1, 2 and 3.

Method of sampling

Thirty fields were sampled, 15 on each of the two drifts, 5 on each of the three soil types. From each field four composite samples were prepared, these representing four successive levels of the first 3 feet, viz., 1-6, 7-12, 13-24, and 25-36 inches. Each is a composite of twenty individual samples taken with an auger, ten of which were from one part of the field and ten from another. The borings of each group of ten were in a straight line and approximately 10 yards apart. Two augers, one 2.0 and the other 1.5 inches in diameter, were used, the larger to take the surface section and to enlarge the hole preparatory to taking each of the lower sections with the smaller auger. We were careful to prevent the soil from the lower sections becoming contaminated with surface soil as the auger was being withdrawn. The composites made up from the twenty borings are referred to as the "field samples," while the "drift samples" from each type were prepared by combining equal weights of the five corresponding "field samples." Thus the drift samples are composites from 100 borings scattered over a considerable territory.

In the forest fields the surface coating of leaf-mold in nearly all cases had become so modified from allowing cattle to pasture in the woods that it was not considered representative of the original leaf-mold. Hence in taking the samples from this type the surface at each boring was freed of all leaves and woody fragments before the augers were used.

TEXTURE

Proportion of coarser particles

As the samples were being taken all rock fragments less than 25 mm. in diameter brought up by the auger were included in the sample and wherever a fragment exceeding this size was encountered another boring was made a foot or so distant, the material from the first being rejected. In the case of all field samples we determined the coarser gravel, the portion included within the limits 2-25 mm. (table 1), and the average weight of its particles (table 2). More detail on this as well as on various other parts of the study has already been reported (15). On glacial areas such as these the proportion of material coarser than 25 mm. in diameter will vary considerably from place to place and its determination would involve the handling and separation in the field of large amounts of soil and subsoil.

On the forest type the average proportion of gravel in the four sections, or in the 20 samples, was much the same on both drifts, 2 per cent on the old and 1.83 per cent on the young, while the average weight of the particles was equally similar, viz., 0.030 and 0.026 gm., respectively. On both the maximum was found in the third foot. Although the variation from field to field on both is so great that we should not attach any great weight to the slight differences shown by the two drifts, it is worthy of note that the difference between the

TABLE 1
Coarser gravel in the successive levels of the thirty fields

DEPTH	OLD DRIFT						YOUNG DRIFT					
	Field I	Field II	Field III	Field IV	Field V	Average	Field I	Field II	Field III	Field IV	Field V	Average
Forest												
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	0.93	0.33	0.53	0.03	2.03	0.77	0.47	1.07	0.64	1.79	0.46	0.89
7-12	1.02	0.31	0.63	0.13	0.03	0.42	0.94	0.75	0.27	1.65	0.26	0.77
13-24	2.52	1.28	1.93	0.19	0.02	1.19	1.52	1.70	1.10	4.25	0.18	1.75
25-36	6.22	6.48	8.24	0.04	0.08	4.21	1.81	2.93	2.90	4.92	1.98	2.91
Average 1-36*	3.23	2.69	3.58	0.10	0.38	2.00	1.34	1.85	1.48	3.63	0.94	1.83
Upland Prairie												
1-6	0.06	0.52	0.23	0.15	0.12	0.22	0.17	1.18	1.00	1.03	0.99	0.88
7-12	0.04	0.68	0.33	0.21	0.50	0.35	0.51	1.08	1.25	1.38	0.98	1.04
13-24	0.13	0.37	0.31	0.13	0.14	0.22	1.32	0.91	2.83	1.91	2.38	1.87
25-36	1.08	0.77	1.15	1.06	1.15	1.04	1.94	2.85	3.44	2.59	4.32	3.03
Average 1-36	0.42	0.58	0.55	0.46	0.53	0.51	1.20	1.63	2.46	1.90	2.56	1.95
Lowland Prairie												
1-6	0.00	0.35	0.00	0.00	0.00	0.07	0.67	0.23	0.64	0.58	0.46	0.52
7-12	0.00	0.17	0.00	0.70	0.07	0.19	1.00	0.23	1.06	1.44	0.83	0.91
13-24	0.00	0.16	0.20	0.27	0.36	0.20	0.94	0.79	1.51	4.28	0.68	1.64
25-36	0.36	2.21	0.17	0.85	0.82	0.88	1.65	1.06	2.07	4.38	1.57	2.15
Average 1-36	0.12	0.88	0.12	0.49	0.40	0.40	1.14	0.69	1.48	3.22	0.96	1.50

* To secure the average for a 3-foot section the mean of the values for the two 6-inch sections is added to those for the second and third feet and the sum divided by three, a procedure followed in all tables reported in this article.

TABLE 2
Average weight of particles of coarser gravel in the six groups of fields

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
<i>inches</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1-6	0.022	0.029	0.025	0.039	0.002	0.022
7-12	0.024	0.020	0.035	0.026	0.019	0.020
13-24	0.032	0.025	0.021	0.024	0.053	0.026
25-36	0.035	0.028	0.033	0.024	0.046	0.023
Average 1-36	0.030	0.026	0.028	0.027	0.036	0.023

third foot and the overlying sections is generally more marked on the Old Drift, as though the disintegration of the original rock fragments had proceeded to a greater depth on this.

The topography of this type is the most rolling of any sampled and accordingly its surface soil is the most apt to be strongly eroded; hence it is not unlikely that much of the material originally at the surface on some of the fields has been carried away, bringing less weathered layers within the 3-foot section.

A mineralogical examination of the sand and gravel in the third foot-section on the two drifts showed that on the older considerably larger quantities of trap-rocks and iron minerals (limonite, magnetite) were present than on the younger while on the latter a higher percentage of quartz, light-colored quartzite, chert, and shales was observed, no trace of the last-named two rocks being found on the older.

On both the upland and the lowland prairie types the proportion of coarse gravel is appreciably higher on the younger drift, 1.95 compared with 0.51 per cent, and 1.50 with 0.40, respectively, as though the processes of weathering had advanced farther on the older. Less variation than on the forest type is shown from field to field. The average weight of the particles is alike on the upland prairie but on the lowland prairie it is the higher in the older drift. The differences between the averages for the two groups of lowland prairie fields is due chiefly to the large amounts of gravel found in field IV on the younger drift.

Thus on the whole the two drifts show no really characteristic differences in the proportion of coarse gravel in the 3-foot section, while the average weight of the particles is much the same from type to type and drift to drift.

Moisture equivalents

When a large number of samples are being compared in texture it is most convenient and often most satisfactory to use some single-valued expression directly related to the water-retaining capacity, either the hygroscopic coefficient or the moisture equivalent. In the present instance we determined the moisture equivalent of each sample, under our circumstances this being the more convenient of the two (tables 3 and 4). The values are shown graphically in figure 4.

The forest type shows the greatest variation from field to field as well as the coarsest texture, while the lowland prairie shows the finest texture and the greatest variation within the 3-foot section. In field IV of the latter type on the Old Drift the moisture equivalent of the surface section, 52.1, is practically twice that of the third foot, 25.9.

The averages for the five fields on the different types (table 4) show little variation from drift to drift. The lowland prairie appears to have a finer texture in the first foot on the older, but this is probably to be attributed to

its higher content of organic matter. This similarity in texture from drift to drift in the case of each of the three types still further enhances the desirability of the area and of these soil types for the chemical study reported in the following sections.

TABLE 3
Moisture equivalents of successive levels of the thirty fields

DEPTH	OLD DRIFT						YOUNG DRIFT					
	Field I	Field II	Field III	Field IV	Field V	Average	Field I	Field II	Field III	Field IV	Field V	Average
Forest												
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	26.0	24.4	23.9	27.7	22.8	24.8	25.5	24.1	23.3	21.2	27.7	24.4
7-12	22.8	20.9	21.2	23.9	21.0	22.0	19.0	21.2	20.1	17.3	25.3	20.6
13-24	22.5	22.8	21.4	25.8	20.4	22.6	20.3	24.4	20.8	18.3	25.7	21.9
25-36	19.6	15.3	15.7	19.1	14.9	16.9	19.8	23.2	18.4	18.3	23.0	20.5
Average 1-36	22.2	20.2	19.9	23.6	19.1	21.0	20.8	23.4	20.3	18.6	25.1	21.6
Upland prairie												
1-6	33.3	27.3	32.9	28.7	31.1	30.6	35.3	28.5	26.2	28.8	29.1	29.6
7-12	31.7	27.9	32.1	28.0	30.7	30.1	35.0	24.3	24.2	28.3	26.9	27.7
13-24	28.6	27.3	29.5	26.3	28.8	28.1	33.3	21.6	22.5	26.7	24.5	25.7
25-36	23.8	24.7	25.9	22.5	23.7	24.1	34.3	22.1	23.1	26.4	23.4	25.9
Average 1-36	28.3	26.5	29.3	25.7	27.8	27.5	34.2	23.4	23.6	27.2	25.3	26.7
Lowland prairie												
1-6	44.7	45.5	40.9	52.1	46.5	45.9	40.5	34.5	35.3	34.1	35.1	35.9
7-12	37.8	38.2	36.7	36.8	36.6	37.2	36.8	27.3	37.5	32.2	32.3	
13-24	29.9	29.9	31.9	30.0	28.6	30.1	35.6	26.6	35.6	26.9	26.7	30.3
25-36	24.3	24.5	27.5	25.9	23.3	25.1	35.1	27.8	32.5	26.3	26.0	29.5
Average 1-36	31.8	32.1	32.7	33.4	31.1	32.2	36.4	28.4	34.8	28.4	28.8	31.4

TABLE 4
Moisture equivalents of the six groups of fields

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	24.8	24.4	30.6	29.6	45.9	35.9
7-12	22.0	20.6	30.1	27.7	37.2	32.8
13-24	22.6	21.9	28.1	25.7	30.1	30.3
25-26	16.9	20.5	24.1	25.9	25.1	29.5
Average 1-36	21.0	21.6	27.5	26.7	32.2	31.4

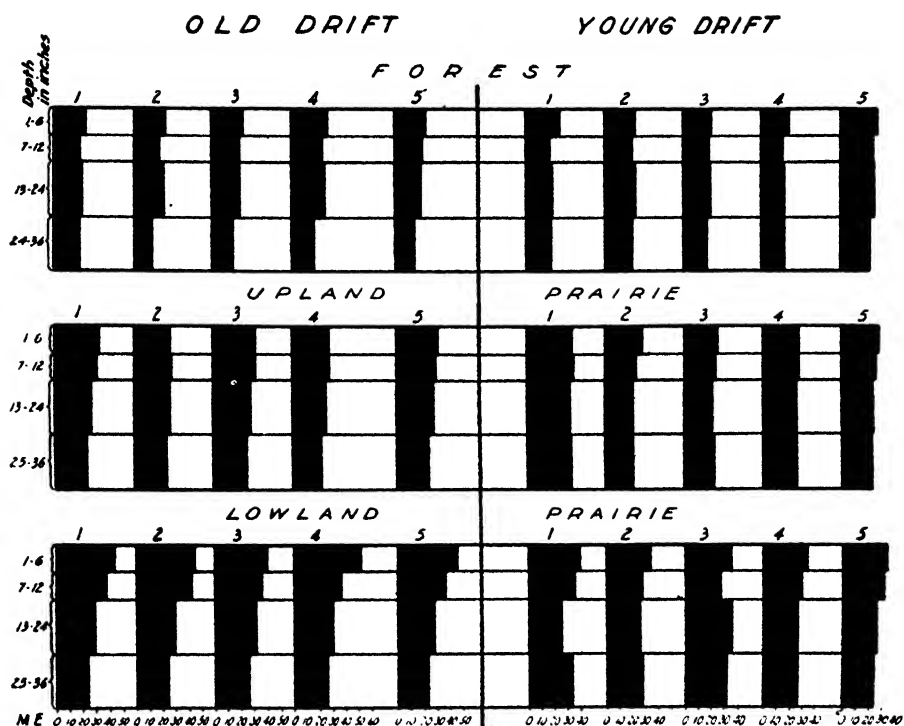


FIG. 4. DIAGRAM SHOWING MOISTURE EQUIVALENTS OF THE SOIL IN THE THIRTY FIELDS

METHODS OF CHEMICAL ANALYSIS

In the case of each of the "drift samples," the composites from the group of five fields of a type on each drift, we made a complete or rock analysis and an organic-carbon determination. With the samples from the individual fields analysis was confined to nitrogen, carbon dioxide, and phosphoric acid, but the reaction and the color were determined, as well as the data reported in tables 1 and 3.

In the complete analysis we followed the methods in use in the laboratory of the United States Geological Survey (7) except in the case of the phosphoric acid for which the modification of the Washington method (17) developed by one of us (Rost) was employed (14).

Nitrogen was determined by the Gunning-Kjeldahl method and organic carbon by combustion with copper oxide in a current of oxygen, while for the reaction the Truog method (16) was used.

The volatile matter was determined by ignition to constant weight and hence includes the carbon dioxide.

The organic matter reported has been computed from the organic carbon— $C \times 1.724 = \text{organic matter}$.

The water of constitution has been obtained by deducting the sum of the organic matter and carbon dioxide from the volatile matter.

The analytical data reported are the averages of concordant duplicate determinations.

For many of the analyses we are indebted to Mr. W. M. Shaw, formerly of this laboratory.

CHEMICAL COMPOSITION

The composition of the 24 drift samples is reported in tables 5, 6 and 7. In the later tables each individual constituent is dealt with separately in order to facilitate the study of the differences between the two drifts, the three types and the four successive levels. Further, in order to bring out as clearly as possible any effect that leaching may have had upon the original constituents other than the carbonates, we report the mineral constituents in tables 8 to 13 upon the basis of the carbonate-free, non-volatile portion of the soil, and not upon the oven-dried samples as in tables 5 to 7. For this reason the percentages in the latter tables must be distinguished from those in the former.

To illustrate our method of computation we may consider the silica in the surface 6-inch section and in the third foot of the lowland prairie on the old drift. The oven-dried samples were found to contain, respectively, 59.05 and 73.41 per cent of silica, 20.34 and 4.08 of volatile matter, and 0.30 and 0.84 of carbon dioxide. Assuming that all the carbon dioxide is present in the form of calcite, the corresponding percentages of this are 0.68 and 1.91. Thus the carbonate-free, non-volatile portion of the two samples constitutes 78.98 and 94.01 per cent of the oven-dried samples, and contains 74.76 and 78.09 per cent of silica. They thus show a difference of only 3.33 per cent compared with 14.36 shown by the ordinary statement of analysis in table 7.

Silica

This constituent is quite similarly distributed on the two drifts, and from type to type (table 8). The highest averages are for the forest and the lowest for the lowland prairie. There is no definite increase or decrease with increase in depth.

Iron

The iron is reported as ferric oxide in table 8-B. Although ferrous compounds were doubtless present no attempt was made to differentiate between ferrous and ferric iron, because of the large amount of organic matter in most of the samples and the interference of this with the determination of the proportion present in the ferrous condition.

All three types show a higher content on the older drift, the differences being least on the forest type. There is a steady increase with depth until the second foot is reached but this does not show any regular difference from the third.

TABLE 5
Composition of forest type (Carrington loam)
 (Data for Young Drift in italics)

	1-6 INCHES	7-12 INCHES	13-24 INCHES	25-36 INCHES	AVERAGE 1-36 INCHES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SiO ₂	75.69 <i>76.34</i>	77.05 <i>77.37</i>	74.95 <i>76.26</i>	78.23 <i>76.98</i>	76.52 <i>76.70</i>
Al ₂ O ₃	9.50 <i>9.52</i>	10.54 <i>10.66</i>	12.01 <i>11.81</i>	10.46 <i>11.77</i>	10.83 <i>11.22</i>
Fe ₂ O ₃	2.93 <i>2.82</i>	3.41 <i>3.32</i>	4.23 <i>3.93</i>	4.04 <i>3.96</i>	3.81 <i>3.65</i>
MgO.....	0.70 <i>0.60</i>	0.71 <i>0.67</i>	0.94 <i>0.84</i>	0.78 <i>0.85</i>	0.81 <i>0.77</i>
CaO.....	1.06 <i>1.09</i>	0.92 <i>0.97</i>	0.97 <i>0.95</i>	1.02 <i>1.07</i>	0.99 <i>1.02</i>
Na ₂ O.....	1.46 <i>1.41</i>	1.42 <i>1.42</i>	1.38 <i>1.40</i>	1.41 <i>1.42</i>	1.41 <i>1.41</i>
K ₂ O.....	1.72 <i>1.86</i>	1.76 <i>1.99</i>	1.82 <i>1.96</i>	1.65 <i>1.76</i>	1.74 <i>1.88</i>
TiO ₂	0.68 <i>0.60</i>	0.73 <i>0.60</i>	0.69 <i>0.58</i>	0.60 <i>0.58</i>	0.67 <i>0.59</i>
P ₂ O ₅	0.23 <i>0.19</i>	0.18 <i>0.15</i>	0.18 <i>0.14</i>	0.15 <i>0.14</i>	0.18 <i>0.15</i>
CO ₂	0.09 <i>0.10</i>	0.04 <i>0.06</i>	0.03 <i>0.05</i>	0.04 <i>0.04</i>	0.06 <i>0.06</i>
Organic C.....	2.83 <i>3.06</i>	1.34 <i>1.46</i>	0.79 <i>0.81</i>	0.50 <i>0.50</i>	1.12 <i>1.19</i>
Organic matter.....	4.87 <i>5.18</i>	2.31 <i>2.52</i>	1.36 <i>1.40</i>	0.86 <i>0.86</i>	1.94 <i>2.04</i>
Water of constitution.....	1.47 <i>1.45</i>	1.37 <i>1.57</i>	1.91 <i>2.33</i>	1.75 <i>1.98</i>	1.69 <i>1.94</i>
N.....	0.24 <i>0.26</i>	0.12 <i>0.12</i>	0.07 <i>0.08</i>	0.04 <i>0.04</i>	0.10 <i>0.10</i>
Volatile matter.....	6.41 <i>6.71</i>	3.73 <i>4.15</i>	3.32 <i>3.77</i>	2.66 <i>2.89</i>	3.68 <i>4.03</i>

TABLE 6
Composition of upland prairie type (Carrington silt loam)
 (Data for Young Drift in italics)

	1-6 INCHES	7-12 INCHES	13-24 INCHES	25-36 INCHES	AVERAGE 1-36 INCHES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SiO ₂	69.59 <i>72.89</i>	71.02 <i>73.62</i>	72.45 <i>75.24</i>	75.14 <i>73.65</i>	72.63 <i>74.05</i>
Al ₂ O ₃	11.09 <i>10.46</i>	11.35 <i>10.87</i>	11.96 <i>11.35</i>	11.89 <i>12.13</i>	11.69 <i>11.38</i>
Fe ₂ O ₃	3.55 <i>2.99</i>	3.81 <i>3.21</i>	4.40 <i>3.42</i>	4.78 <i>3.81</i>	4.29 <i>3.44</i>
MgO.....	0.81 <i>0.72</i>	0.84 <i>0.74</i>	0.85 <i>0.88</i>	0.93 <i>1.21</i>	0.87 <i>0.94</i>
CaO.....	1.13 <i>1.24</i>	1.14 <i>1.18</i>	1.02 <i>1.24</i>	0.93 <i>2.08</i>	1.03 <i>1.51</i>
Na ₂ O.....	1.26 <i>1.39</i>	1.36 <i>1.35</i>	1.37 <i>1.33</i>	1.29 <i>1.31</i>	1.32 <i>1.33</i>
K ₂ O.....	1.76 <i>1.66</i>	1.84 <i>1.74</i>	1.90 <i>1.86</i>	1.87 <i>1.87</i>	1.86 <i>1.80</i>
TiO ₂	0.64 <i>0.50</i>	0.64 <i>0.52</i>	0.64 <i>0.54</i>	0.70 <i>0.53</i>	0.66 <i>0.53</i>
P ₂ O ₅	0.23 <i>0.18</i>	0.20 <i>0.17</i>	0.15 <i>0.14</i>	0.13 <i>0.11</i>	0.16 <i>0.14</i>
CO ₂	0.08 <i>0.07</i>	0.09 <i>0.05</i>	0.06 <i>0.13</i>	0.03 <i>1.06</i>	0.06 <i>0.42</i>
Organic C.....	4.76 <i>4.48</i>	3.77 <i>3.19</i>	1.83 <i>1.78</i>	0.75 <i>0.77</i>	2.28 <i>2.13</i>
Organic matter.....	8.20 <i>7.72</i>	6.50 <i>5.50</i>	3.15 <i>3.07</i>	1.29 <i>1.33</i>	3.93 <i>3.67</i>
Water of constitution.....	2.34 <i>1.58</i>	2.00 <i>2.25</i>	2.86 <i>2.14</i>	2.41 <i>2.86</i>	2.46 <i>2.30</i>
N.....	0.38 <i>0.36</i>	0.29 <i>0.28</i>	0.17 <i>0.16</i>	0.06 <i>0.07</i>	0.19 <i>0.08</i>
Volatile matter.....	10.52 <i>9.38</i>	8.58 <i>7.80</i>	6.07 <i>5.34</i>	3.73 <i>5.25</i>	6.54 <i>6.39</i>

TABLE 7
Composition of lowland prairie type (Fargo silt loam)
 (Data for young drift in italics)

	1-6 INCHES	7-12 INCHES	13-24 INCHES	25-36 INCHES	AVERAGE 1-36 INCHES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SiO ₂	{ 59.05 68.08	{ 65.81 70.42	{ 71.71 72.02	{ 73.41 71.97	{ 69.18 71.08
Al ₂ O ₃	{ 10.29 10.18	{ 11.46 10.62	{ 12.43 11.57	{ 12.28 12.64	{ 11.86 11.50
Fe ₂ O ₃	{ 3.37 3.21	{ 3.79 3.21	{ 4.21 3.68	{ 4.14 3.95	{ 3.98 3.61
MgO.....	{ 1.02 1.07	{ 1.06 1.08	{ 1.21 1.23	{ 1.49 1.51	{ 1.25 1.27
CaO.....	{ 2.34 1.97	{ 2.08 1.84	{ 1.55 1.86	{ 1.92 2.16	{ 1.89 1.97
Na ₂ O.....	{ 1.31 1.18	{ 1.48 1.29	{ 1.45 1.27	{ 1.59 1.18	{ 1.48 1.23
K ₂ O.....	{ 1.57 1.60	{ 1.64 1.68	{ 1.83 1.71	{ 1.86 1.75	{ 1.76 1.70
TiO ₂	{ 0.60 0.60	{ 0.66 0.60	{ 0.73 0.60	{ 0.72 0.60	{ 0.69 0.60
P ₂ O ₅	{ 0.31 0.23	{ 0.24 0.18	{ 0.19 0.15	{ 0.18 0.11	{ 0.21 0.16
CO ₂	{ 0.30 0.49	{ 0.20 0.57	{ 0.06 0.54	{ 0.84 1.04	{ 0.38 0.70
Organic C.....	{ 9.47 5.71	{ 5.38 3.91	{ 1.66 2.10	{ 0.62 1.00	{ 3.23 2.64
Organic matter.....	{ 16.32 9.84	{ 9.27 6.74	{ 2.86 3.62	{ 1.07 1.72	{ 5.57 4.54
Water of constitution.....	{ 2.72 1.35	{ 2.66 2.30	{ 2.49 2.27	{ 1.17 1.36	{ 2.62 2.40
N.....	{ 0.79 0.44	{ 0.47 0.31	{ 0.15 0.15	{ 0.04 0.06	{ 0.27 0.19
Volatile matter.....	{ 20.34 11.68	{ 12.12 9.61	{ 5.41 6.43	{ 4.08 5.87	{ 8.57 7.31

TABLE 8
Silica, ferric oxide, alumina and titanium in the different sections

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift

A. Silica						
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	81.04	82.03	77.93	80.56	74.76	78.07
7-12	80.11	80.84	77.84	79.95	75.28	79.03
13-24	77.58	79.34	77.24	79.73	75.92	77.98
25-36	80.45	79.34	78.10	77.93	78.09	78.42
Average 1-36	79.52	80.01	77.74	79.30	76.24	78.27

B. Ferric oxide						
1-6	3.14	3.03	3.97	3.30	4.26	3.68
7-12	3.55	3.46	4.17	3.48	4.33	3.60
13-24	4.38	4.09	4.69	3.62	4.46	3.98
25-36	4.15	4.08	4.97	4.03	4.40	4.30
Average 1-36	3.96	3.87	4.58	3.67	4.37	3.97

C. Alumina						
1-6	10.17	10.22	12.40	11.56	13.03	11.67
7-12	10.96	11.14	12.44	11.80	13.10	11.91
13-24	12.43	12.28	12.75	12.02	13.16	12.52
25-36	10.76	12.12	12.35	12.82	13.06	13.67
Average 1-36	11.25	11.70	12.51	12.17	13.09	12.66

D. Titanium oxide						
1-6	0.73	0.64	0.71	0.55	0.75	0.69
7-12	0.76	0.63	0.70	0.56	0.75	0.67
13-24	0.71	0.60	0.68	0.57	0.77	0.65
25-36	0.62	0.60	0.73	0.55	0.76	0.65
Average 1-36	0.69	0.61	0.71	0.56	0.75	0.66

Alumina

The alumina (table 8-C) is quite uniformly distributed on the two drifts as well as from type to type, although slightly higher in the prairie fields on the older drift, but on this it shows a minimum of 10.17 and a maximum of 13.16 per cent and on the younger a range from 10.22 to 13.67 per cent. As in the case of the ferric oxide, there is an increase with depth through the first three sections but the second foot is not distinctly higher than the third. The similarity in the alumina content on the two drifts is evident from the averages for the 3-foot sections of all three types, viz., 12.28 per cent on the older and 12.18 per cent on the younger.

Titanium

The titanium (table 8-D) although slightly higher on the older drift is very uniformly distributed, both from type to type and from the surface downward.

Lime

The total lime (table 9-A) in the different sections of the forest type is remarkably similar, the differences between the two drifts for any given level being no greater than that between duplicate determinations on the same sample. All calcium compounds at all readily soluble have evidently been

TABLE 9
Lime in the different sections

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
A. Total lime						
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	1.13	1.17	1.26	1.37	2.96	2.26
7-12	0.96	1.01	1.25	1.28	2.37	2.06
13-24	1.00	0.99	1.09	1.31	1.64	2.01
25-36	1.05	1.10	0.97	2.20	2.02	2.33
Average 1-36	1.03	1.06	1.10	1.61	2.11	2.17
B. Lime in the form of carbonate as computed from CO ₂ content						
1-6	0.09	0.10	0.10	0.10	0.40	0.65
7-12	0.07	0.08	0.10	0.07	0.25	0.69
13-24	0.07	0.05	0.07	0.16	0.08	0.68
25-36	0.06	0.07	0.04	1.36	1.06	1.33
Average 1-36	0.07	0.07	0.07	0.53	0.49	0.89
C. Lime in form of silicate (= A - B)						
1-6	1.04	1.07	1.16	1.27	2.56	1.61
7-12	0.89	0.93	1.15	1.21	2.12	1.37
13-24	0.93	0.94	1.02	1.15	1.56	1.33
25-36	0.99	1.03	0.93	0.84	0.96	1.00
Average 1-36	0.96	0.99	1.03	1.08	1.62	1.27

leached out of this type to a depth of more than 3 feet. On the upland prairie the amount in the first three sections is practically the same on both drifts, varying between 1.09 and 1.37 per cent. In the third foot, however, the quantity on the younger drift is more than twice as great, reaching 2.20 per cent, as compared with 0.97 on the older. On the lowland prairie there is no wide difference between the drifts, and the average for the three feet is 2.11 per cent in the case of the older and 2.17 in the younger.

The total lime in the surface section is generally slightly higher than in the second. It is withdrawn from the lower levels by plant roots, being largely deposited in the leaves and branches, on the death and decay of which it remains in the surface layer.

The amount of lime present as carbonate (table 9-B) has been computed from the carbon-dioxide content, assuming that all of this is present in the form of calcite. An appreciable quantity is shown on the upland prairie type only in the third foot-section of the Young Drift. A deficiency in this is shown in the second foot-section on the lowland prairie only on the Old Drift. With the forest type on both drifts the carbonate has been leached to a depth greater than 3 feet. On the older drift the two types, and on the younger the forest, have lost the more readily soluble calcium compounds to a depth of more than 3 feet. The upland prairie on the younger still retains a considerable amount in the third foot. Any serious leaching of the lowland prairie has been prevented by the poorly drained condition, as is well illustrated by table 9-B, which shows every section well supplied with carbonate except the second foot on the older drift.

Deducting the lime in the form of carbonate from the total gives the amount in the form of silicate (table 9-C), assuming that the quantity of gypsum is negligible. On each type the average amount is very similar on the two drifts except in the case of the lowland prairie, where it is slightly the higher on the older. On the prairie types from the surface downward, there is a general decrease in the amount of non-carbonate lime, while on the forest type there is but little variation.

The similarity in carbon-dioxide content from field to field is dealt with in a later section (tables 21 and 22).

Magnesia

There is very little difference between the two drifts in the amount of magnesia (table 10) found on any of the types. As in the case of lime, the most occurs in the lowland prairie, the average being 1.35 per cent for the Old Drift and 1.39 on the Young. For the forest the averages are slightly lower than for upland prairie, being 0.84 per cent and 0.80 per cent, respectively for the two drifts. The magnesia content rises with increasing depth.

Ratio of lime to magnesia

The ratio of total lime to magnesia (table 11-A) falls between 1.0 and 1.8, averaging 1.4 for the 3-foot section on all types. For each of these it is very similar on both drifts, the greatest difference being shown by the upland prairie, where, on the older drift, it is 1.2 as compared with 1.5 on the younger.

On both prairie types the ratio of non-carbonate lime to magnesia decreases from the surface downward (table 11-B), partly because of the similar decrease

in the non-carbonate lime and a parallel increase in the magnesia. On the forest type the ratio decreases through only the first three sections, rising slightly in the third foot.

TABLE 10
Magnesia in the different sections

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	0.75	0.64	0.91	0.80	1.29	1.23
7-12	0.74	0.70	0.92	0.80	1.21	1.21
13-24	0.97	0.87	0.91	0.93	1.28	1.33
25-36	0.80	0.87	0.97	1.28	1.57	1.63
Average 1-36	0.84	0.80	0.93	1.00	1.35	1.39

TABLE 11
Relation of lime to magnesia in the different sections

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
A. Ratio of total lime to magnesia						
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	1.5	1.8	1.4	1.7	2.3	1.8
7-12	1.3	1.4	1.3	1.6	2.0	1.7
13-24	1.0	1.1	1.2	1.4	1.3	1.5
25-36	1.3	1.3	1.0	1.7	1.3	1.4
Average 1-36	1.2	1.3	1.2	1.5	1.6	1.6
B. Ratio of lime in form of silicate to magnesia						
1-6	1.4	1.6	1.2	1.5	2.0	1.3
7-12	1.2	1.3	1.2	1.5	1.7	1.1
13-24	1.0	1.0	1.1	1.2	1.2	1.0
25-36	1.2	1.2	0.9	0.6	0.6	0.6
Average 1-36	1.2	1.2	1.1	1.1	1.2	0.9

Potash

The distribution of potash (table 12-A) is fairly uniform for each type, the averages for the 3-foot section being practically the same for the two drifts, with the exception of the forest, which shows slightly higher amounts on the younger drift, averaging 1.96 per cent compared with 1.81 on the older. The average for the 3-foot section of all three types is 1.91 per cent for the older and 1.93 for the younger. The proportion shows no dependence upon the depth of the section.

Soda

Except on the lowland prairie the soda (table 12-B) shows no difference between drifts and but little variation with depth. For the entire 3-foot section the average content on the forest and upland prairie types is alike for both drifts.

On the lowland prairie it is considerably higher at all levels on the older drift, on which it is higher than on either the upland prairie or the forest fields, while on the younger drift it is lower than on either of these.

TABLE 12
Potash and soda in the different sections

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
A. Potash						
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	1.84	2.00	1.97	1.83	1.99	1.84
7-12	1.83	2.08	2.02	1.89	1.87	1.88
13-24	1.88	2.04	2.02	1.97	1.94	1.85
25-36	1.70	1.81	1.94	1.98	1.96	1.90
Average 1-36	1.80	1.96	1.98	1.93	1.94	1.87
B. Soda						
1-6	1.56	1.51	1.41	1.54	1.66	1.36
7-12	1.48	1.48	1.49	1.47	1.69	1.45
13-24	1.42	1.46	1.46	1.41	1.53	1.37
25-36	1.45	1.46	1.34	1.38	1.68	1.27
Average 1-36	1.46	1.47	1.42	1.43	1.62	1.34

Phosphoric acid

In all six groups phosphoric acid shows a decrease from the surface downward and with each type is the higher on the older drift and on both drifts it is highest in the lowland prairie (table 13).

This constituent was determined in the four sections from each of the 30 fields—120 samples in all (table 14 and fig. 5). From these it will be seen that there is less regularity than where composites of a large number of samples are used. In the forest fields the vertical distribution is more or less irregular, although the maximum in every field is found in the surface section, but on the upland prairie each of the ten fields shows the decrease with depth, and on the lowland prairie only two of the ten fail to conform to this regularity. From this it would appear that in the 3-foot section of practically every field on all three types on both drifts the phosphoric acid would show a decrease with depth if the samples analyzed were composites from a large number of

TABLE 13
*Phosphoric acid in the six groups of fields**

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	0.249	0.208	0.252	0.201	0.394	0.259
7-12	0.187	0.154	0.224	0.182	0.278	0.206
13-24	0.182	0.149	0.161	0.143	0.197	0.163
25-36	0.152	0.144	0.134	0.119	0.189	0.120
Average 1-36	0.184	0.158	0.178	0.151	0.241	0.172

* On the basis of the carbonate-free inorganic portion of the soils and not on that of oven-dried soil as in table 14.

TABLE 14
*Phosphoric acid in the different sections from the thirty fields**

DEPTH	OLD DRIFT						YOUNG DRIFT					
	Field I	Field II	Field III	Field IV	Field V	Average	Field I	Field II	Field III	Field IV	Field V	Average
Forest												
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	0.236	0.216	0.220	0.280	0.213	0.233	0.213	0.175	0.207	0.162	0.213	0.194
7-12	0.182	0.160	0.178	0.223	0.156	0.180	0.178	0.140	0.159	0.115	0.143	0.147
13-24	0.150	0.162	0.194	0.197	0.178	0.176	0.162	0.147	0.147	0.131	0.128	0.143
25-36	0.124	0.159	0.134	0.188	0.143	0.150	0.162	0.162	0.110	0.131	0.137	0.140
Average 1-36	0.161	0.170	0.176	0.212	0.168	0.177	0.173	0.155	0.147	0.133	0.148	0.151
Upland prairie												
1-6	0.245	0.220	0.258	0.214	0.204	0.228	0.204	0.182	0.172	0.175	0.178	0.182
7-12	0.200	0.223	0.220	0.191	0.188	0.204	0.210	0.151	0.153	0.172	0.156	0.168
13-24	0.159	0.165	0.147	0.137	0.147	0.151	0.156	0.108	0.144	0.147	0.118	0.135
25-36	0.140	0.143	0.127	0.102	0.134	0.129	0.102	0.104	0.121	0.143	0.096	0.113
Average 1-36	0.174	0.176	0.171	0.147	0.159	0.165	0.155	0.126	0.142	0.154	0.127	0.141
Lowland prairie												
1-6	0.290		0.255	0.363	0.338	0.311	0.239	0.229	0.219	0.220	0.216	0.225
7-12	0.248	0.223	0.207	0.264	0.283	0.243	0.185	0.156	0.213	0.179	0.185	0.184
13-24	0.169	0.182	0.162	0.207	0.210	0.186	0.146	0.172	0.188	0.134	0.115	0.151
25-36	0.179	0.172	0.168	0.201	0.175	0.179	0.118	0.099	0.121	0.112	0.111	0.112
Average 1-36	0.206		0.187	0.240	0.232	0.214	0.159	0.154	0.175	0.148	0.142	0.156

* On basis of oven-dried soil.

borings, although from place to place in the field there may be a departure. In an earlier study of prairie soils we have shown that a decrease in phosphoric acid from the first to the twelfth inch is characteristic of prairie soils (2).

Thus we find a consistent difference between the two drifts in their content of phosphoric acid, the older showing a higher content in each of the four sections on all three soil types. Also the average content in the 3-foot section, when individual fields on the same soil type but on different drifts are compared, is with but few exceptions higher on the older drift.

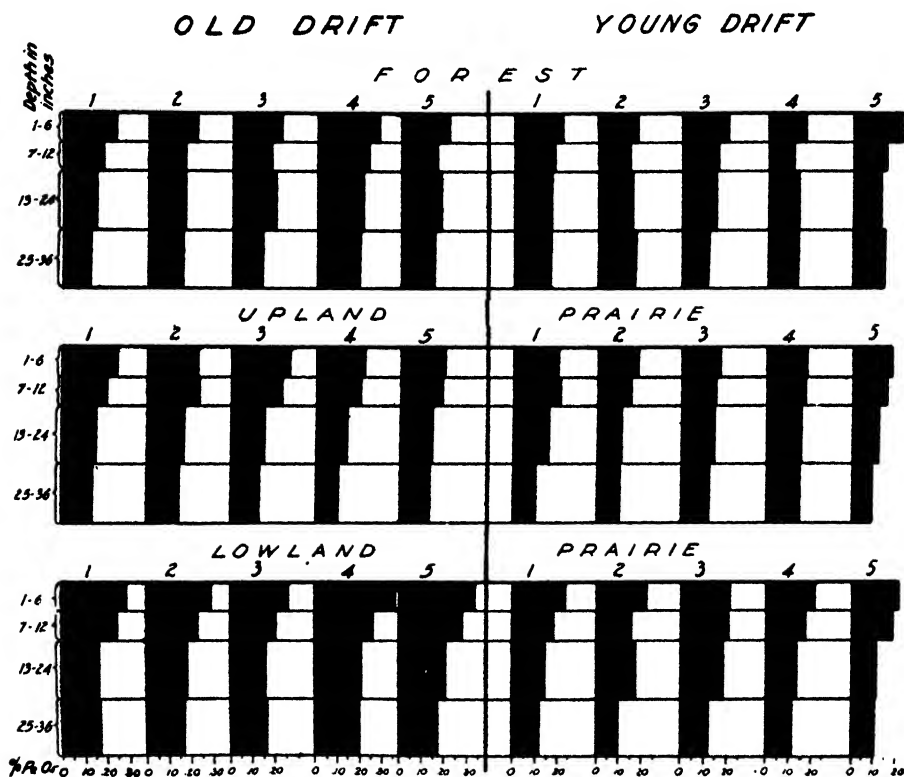


FIG. 5. DIAGRAM SHOWING DISTRIBUTION OF PHOSPHORIC ACID IN THE THIRTY FIELDS

Ratio of phosphoric acid to organic carbon

The concentration of phosphoric acid in the surface layers is connected with both the organic matter still remaining and that which has been converted into gaseous products (2, p. 496).

The relation between the phosphoric acid and the present content of organic matter will be evident from table 15 showing the ratio of organic carbon to total phosphoric acid. The ratio shows a wide range, from 3.3 to 30.4, and decreases rapidly with depth. In the case of the forest and upland prairie soils at all levels the ratio is somewhat higher on the younger drift. This

is due to the lower content of phosphoric acid and not to a greater amount of organic matter in the soil of the younger drift. In the lowland prairie no such regularity in the ratio is shown.

TABLE 15
Ratio of organic carbon to total phosphoric acid in the six groups of fields

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	12.1	15.7	20.9	24.6	30.4	25.3
7-12	7.4	10.0	18.5	19.0	22.1	21.5
13-24	4.4	5.6	12.0	13.2	8.9	13.9
25-36	3.3	4.3	5.8	6.8	3.4	9.0
Average 1-36	5.8	7.6	12.5	13.9	12.8	15.4

Nitrogen

On the forest type the variation in nitrogen from field to field, as well as the distribution from the surface downward, is much the same on the two drifts and there is little difference between the averages for the two.

TABLE 16
Nitrogen in the different sections from the thirty fields

DEPTH	OLD DRIFT						YOUNG DRIFT					
	Field I	Field II	Field III	Field IV	Field V	Average	Field I	Field II	Field III	Field IV	Field V	Average
Forest												
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	0.276	0.242	0.230	0.259	0.210	0.243	0.345	0.152	0.287	0.183	0.340	0.263
7-12	0.192	0.083	0.121	0.135	0.080	0.122	0.151	0.058	0.138	0.067	0.173	0.117
13-24	0.133	0.045	0.059	0.063	0.062	0.072	0.084	0.040	0.126	0.060	0.109	0.084
25-36	0.062	0.043	0.027	0.033	0.031	0.039	0.048	0.034	0.050	0.041	0.047	0.044
Average 1-36	0.143	0.083	0.087	0.098	0.079	0.098	0.128	0.060	0.129	0.075	0.137	0.106
Upland prairie												
1-6	0.458	0.318	0.389	0.362	0.390	0.383	0.437	0.366	0.315	0.314	0.386	0.364
7-12	0.300	0.273	0.291	0.265	0.331	0.292	0.379	0.210	0.272	0.264	0.265	0.278
13-24	0.147	0.164	0.181	0.149	0.187	0.166	0.234	0.174	0.131	0.140	0.143	0.164
25-36	0.050	0.064	0.061	0.052	0.063	0.058	0.083	0.053	0.063	0.065	0.068	0.066
Average 1-36	0.192	0.174	0.194	0.171	0.203	0.187	0.242	0.172	0.162	0.165	0.179	0.184
Lowland prairie												
1-6	0.855	0.505	0.605	1.012	0.955	0.786	0.525	0.427	0.396	0.391	0.484	0.445
7-12	0.518	0.477	0.401	0.397	0.538	0.466	0.320	0.212	0.407	0.261	0.324	0.305
13-24	0.165	0.139	0.167	0.131	0.157	0.152	0.107	0.085	0.316	0.102	0.128	0.148
25-36	0.042	0.050	0.058	0.028	0.043	0.044	0.047	0.034	0.143	0.060	0.040	0.065
Average 1-36	0.298	0.227	0.242	0.288	0.315	0.274	0.192	0.146	0.287	0.163	0.191	0.196

On the upland prairie considerable variation from field to field is shown but this is similar on the two drifts and the averages for the five fields on each are very similar (tables 16 and 17, and fig. 6).

TABLE 17
*Difference in nitrogen content of the three soil types**

DEPTH	FOREST			UPLAND PRAIRIE			LOWLAND PRAIRIE		
	Old drift	Young drift	Difference	Old drift	Young drift	Difference	Old drift	Young drift	Difference
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	0.243	0.263	0.020	0.383	0.364	0.019	0.786	0.445	0.341
7-12	0.122	0.117	0.005	0.292	0.278	0.014	0.466	0.305	0.161
13-24	0.072	0.084	0.012	0.166	0.164	0.002	0.152	0.148	0.004
25-36	0.039	0.044	0.005	0.058	0.066	0.008	0.044	0.065	0.021
Average 1-36	0.098	0.106	0.008	0.187	0.184	0.003	0.274	0.196	0.078

* Wherever the older drift is the richer the difference is shown in italics.

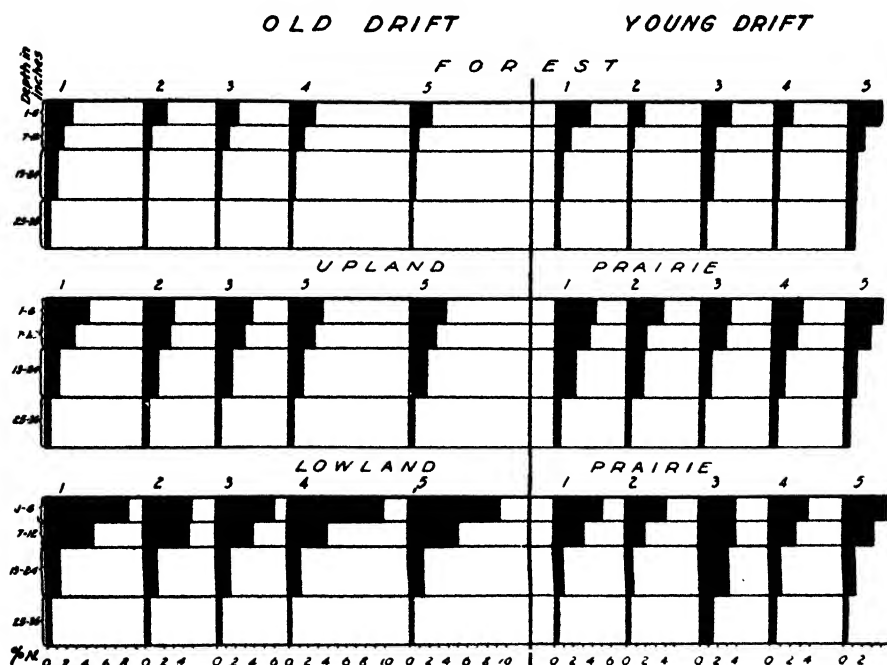


FIG. 6. DIAGRAM SHOWING DISTRIBUTION OF NITROGEN IN THE THIRTY FIELDS

On the lowland prairie fields which were in general far the richest in nitrogen a wide difference in the amounts of nitrogen was found in the surface foot on the two drifts, those on the older averaging 76 per cent the higher in the first 6 inches and 52 in the second. The conditions under which the soils of this type were developed, dealt with in the next section, furnish a possible expla-

nation for this difference, the accumulation of organic matter and nitrogen being parallel. The average amounts of nitrogen found in the second and third foot-sections on the two drifts are quite similar.

Organic carbon

The organic carbon in the 24 group composites was determined by combustion with copper oxide in a current of oxygen (table 18). In order to decompose any carbonates present the samples were first moistened with phosphoric-acid solution and evaporated to dryness.

The differences in organic carbon agree with those in nitrogen. There is but little with the forest soils while with the prairies the old drift is the richer in the first two sections, the difference being especially marked in the lowland type. This may be attributed to obstruction of the drainage on the older

TABLE 18
Difference in organic carbon in the three soil types

DEPTH	FOREST			UPLAND PRAIRIE			LOWLAND PRAIRIE		
	Old drift	Young drift	Difference	Old drift	Young drift	Difference	Old drift	Young drift	Difference
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	2.83	3.06	0.23	4.76	4.48	0.28	9.47	5.71	3.76
7-12	1.34	1.46	0.12	3.77	3.19	0.58	5.38	3.91	1.47
13-24	0.79	0.81	0.02	1.83	1.78	0.05	1.66	2.10	0.44
25-36	0.50	0.50	0.00	0.75	0.77	0.02	0.62	1.00	0.38
Average 1-36	1.12	1.19	0.07	2.28	2.13	0.15	3.23	2.64	0.59

* Wherever the older drift is the richer the difference is shown in italics.

drift, due to the advance of the last ice sheet. The course of the streams before the Wisconsin glaciation was in a general southwesterly direction, but first the ice, and later the drift it left behind, blocked the previously existing drainage channels and forced the water to find outlets to the southeast. Before these new channels were fully developed the drainage was very incomplete and undoubtedly large areas were covered by standing water part of the season and surface material eroded from the surrounding higher land was deposited on the lowlands. Organic material would accumulate also through the growth of dense lowland vegetation and the deposition of its remains.

Little or no peat now remains on the older drift, and because of the shallowness of the water perhaps but little ever formed on it. On the younger drift the poorly drained areas are not so numerous as on the older and such as exist are more in the form of deep "pot holes" in which the conditions for the formation of peat have been favorable. So on this we usually find the lowland prairie forming a narrow band between the bog and the upland prairie.

If at the time of the retreat of the last ice sheet part of the organic carbon now found in the surface soil of the lowland prairie was derived from the eroded surface of the surrounding high land, this would account for some of the differences now found. The surface of the older drift, undisturbed by the Wisconsin ice, would carry a surface soil more or less rich in organic carbon while the material of the till freshly exposed by the retreating ice front would carry very little. Accordingly, the eroded material deposited on the lower-lying levels of older drift would be much richer in organic matter than that on the younger.

Ratio of organic carbon to nitrogen

The ratio of organic carbon to nitrogen shows no regular difference from drift to drift (table 19).

TABLE 19
Ratio of organic carbon to nitrogen in the three soil types

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	11.6	11.6	12.4	12.3	12.0	12.8
7-12	11.0	12.4	12.9	11.4	11.5	12.8
13-24	11.0	9.6	11.0	10.8	10.9	14.1
25-36	12.9	11.3	12.9	11.6	14.1	15.3
Average 1-36	11.7	10.3	12.1	11.4	12.2	14.1

Water of constitution

The water of constitution shows no dependence upon the age of the drift. A statement of the volatile matter of the soil has in itself very little significance, but when once the organic carbon has been determined the organic matter may be computed with a fair degree of reliability by the formula given above, viz., organic matter = organic C \times 1.724, and the difference between the volatile matter and the sum of the organic matter so determined and the carbon dioxide expresses the water of constitution, the water not expelled at 110°C. but driven off below a dull red heat. As this value is found by difference any errors in the determination of the organic carbon or carbon dioxide will be reflected in the percentage found for the water of constitution.

Color

Comparisons were made of the colors of all the samples. For this purpose 25-gm. portions of the fine earth were placed in small porcelain dishes, moistened and allowed to stand for an hour. Then all the moistened samples were arranged in order of color, the darkest being placed at one end and the lightest-colored at the other.

No distinct and characteristic difference between the drifts was found except that in the third foot of the upland prairie the fields on the older drift had a distinctly reddish tint in contrast with the gray or yellowish gray of the younger (15, p. 59). On the forest and lowland prairie fields no characteristic difference was observed—the reddish tint being absent even from

TABLE 20
Volatile matter, organic matter, and water of constitution of the three soil types

DEPTH	FOREST		UPLAND PRAIRIE		LOWLAND PRAIRIE	
	Old drift	Young drift	Old drift	Young drift	Old drift	Young drift
A. Volatile Matter*						
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-6	6.41	6.71	10.52	9.38	20.34	11.68
7-12	3.73	4.15	8.58	7.80	12.12	9.61
13-24	3.32	3.77	6.07	5.34	5.41	6.43
25-36	2.66	2.89	3.73	5.25	4.08	5.87
Average 1-36	3.68	4.03	6.45	6.39	8.57	7.31
B. Organic matter ($C \times 1.724$)						
1-6	4.87	5.18	8.20	7.72	16.32	9.84
7-12	2.31	2.52	6.50	5.50	9.27	6.74
13-24	1.36	1.40	3.15	3.07	2.86	3.62
25-36	0.86	0.86	1.29	1.33	1.07	1.72
Average 1-36	1.94	2.04	3.93	3.67	5.57	.54
C. Water of constitution						
1-6	1.47	1.45	2.24	1.58	3.72	1.35
7-12	1.37	1.57	2.00	2.25	2.66	2.30
13-24	1.91	2.33	2.86	2.14	2.49	2.27
25-36	1.75	1.98	2.41	2.86	2.17	3.11
Average 1-36	1.69	1.94	2.46	2.30	2.62	2.40

* CO_2 included in volatile matter.

the third foot. So far as the intensity of the dark color is concerned the only distinct difference between the two drifts observed was its somewhat more pronounced development on the younger drift in the case of the surface section of the upland prairie, just the opposite to that reported by Burke and Kolbe, who state that "The Kansan drift has generally a darker color than the Wisconsin" (4, p. 21).

Reaction and calcareousness

In the case of all the field samples the reaction was ascertained by the Truog method and the carbon-dioxide content determined (tables 21 and 22). Wherever a sample gave a distinctly acid reaction the carbonate content was

TABLE 21

Calcareousness—degree of acidity (a) of acid samples and carbonate content (b) of others

DEPTH	OLD DRIFT					YOUNG DRIFT				
	Field I	Field II	Field III	Field IV	Field V	Field I	Field II	Field III	Field IV	Field V
Forest										
<i>inches</i>										
1-6	med.	str.	str.	str.	med.	sl.	sl.	med.	sl.	str.
7-12	med.	str.	med.	str.	str.	sl.	sl.	str.	sl.	med.
13-24	sl.	str.	str.	med.	str.	med.	sl.	str.	sl.	med.
25-36	sl.	v.sl.	med.	sl.	str.	med.	sl.	med.	v.sl.	sl.
Upland prairie										
1-6	med.	str.	med.	str.	med.	med.	sl.	med.	med.	med.
7-12	med.	med.	med.	str.	med.	med.	v.sl.	med.	med.	sl.
13-24	str.	med.	str.	v.str.	str.	sl.	0.39	0.79	sl.	sl.
25-36	sl.	med.	med.	str.	med.	v.sl.	3.55	7.12	v.sl.	1.23
Lowland prairie										
1-6	sl.	0.19	med.	0.32	2.10	5.06	med.	med.	sl.	sl.
7-12	sl.	0.18	sl.	0.20	1.52	5.47	sl.	sl.	sl.	sl.
13-24	0.09	0.12	v.sl.	0.01	0.36	5.67	v.sl.	v.sl.	v.sl.	v.sl.
25-36	0.18	1.11	2.27	0.02	5.82	9.05	0.24	0.08	2.35	0.04

(a) v.sl. = very slight; sl. = slight; med. = medium; str. = strong; v.str. = very strong, as determined by the Truog method.

(b) Computed from CO₂ as per cent CaCO₃.

TABLE 22

Carbon dioxide obtained from foot sections of 15 fields, all of which were acid throughout the 3-foot profile

DEPTH	OLD DRIFT						YOUNG DRIFT					
	Field I	Field II	Field III	Field IV	Field V	Average	Field I	Field II	Field III	Field IV	Field V	Average
Forest												
<i>foot</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.064	0.048	0.062	0.070	0.072	0.063	0.098	0.078	0.069	0.048	0.064	0.071
2	0.032	0.084	0.006	0.064	0.080	0.053	0.048	0.036	0.040	0.044	0.044	0.042
3	0.046	0.076	0.020	0.056	0.060	0.051	0.026	0.050	0.036	0.108	0.042	0.052
Average	0.047	0.069	0.029	0.063	0.071	0.056	0.057	0.055	0.048	0.067	0.050	0.055
Upland prairie												
1	0.086	0.064	0.074	0.092	0.082	0.079						
2	0.045	0.056	0.075	0.064	0.046	0.057						
3	0.031	0.035	0.036	0.016	0.024	0.034						
Average	0.054	0.052	0.062	0.057	0.051	0.057						

found negligible, but the reverse does not hold true, a few of the neutral samples showing an equally small content of carbon dioxide.

The carbon dioxide obtained from samples with an acid reaction is not to be attributed to contained carbonate but to the decomposition of organic matter. In nearly every field in which all four samples from the 3-foot section gave an acid reaction the most carbon dioxide was obtained from the surface section, the one richest in organic matter, although this was in general the most acid, as is well illustrated by table 22.

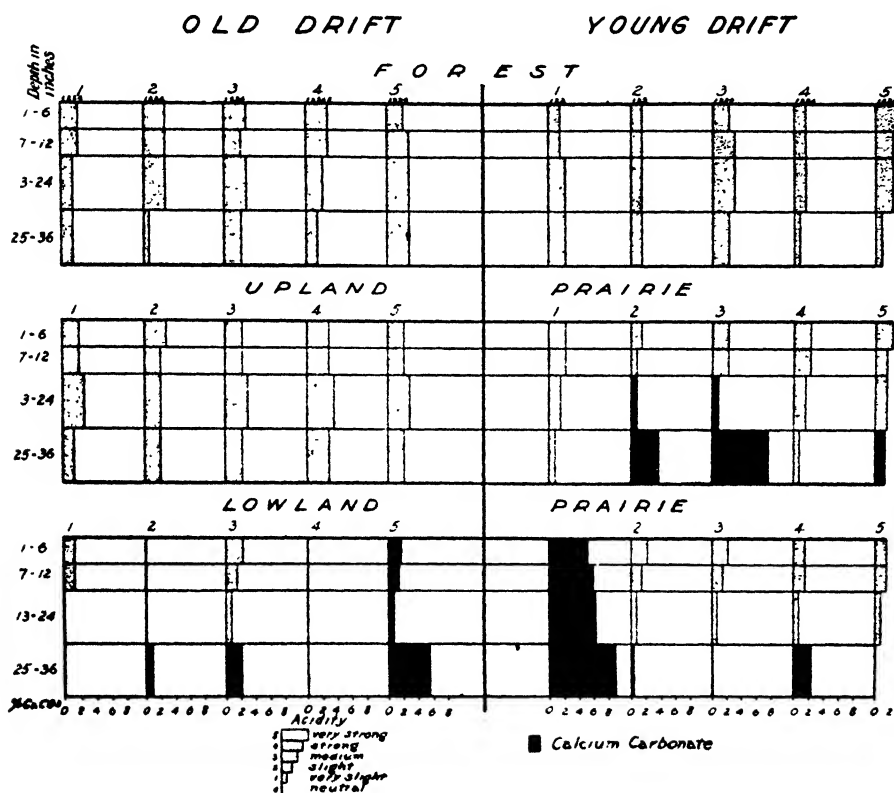


FIG. 7. DIAGRAM SHOWING THE REACTION AND CARBONATE CONTENT IN THE THIRTY FIELDS

While both the reaction and the carbon-dioxide content were determined in the case of every sample, the lime supply is best indicated in the form of the degree of acidity in the case of the acid samples and the amount of carbonate, computed as calcium carbonate, in those of neutral reaction (fig. 7).

With the forest type no distinct difference between drifts was shown, all four levels from the 3-foot section in the ten fields showing an acid reaction, but on the upland prairie there was, on the whole, a better supply of carbonate on the younger drift. The subsoil in three fields, no. II, III and V, on the latter showed a neutral reaction and an appreciable amount of carbonate,

while in all the fields on the older drift it was acid. The greater degree of leaching in the forest type on the Young Drift, compared with the upland prairie of the same age, is to be attributed to the coarser texture of the former, permitting more rapid percolation and causing less water from the rain and snow to be retained in the surface layers and later returned to the air by transpiration and direct evaporation.

In the case of the upland prairie the difference in carbonate content between the two drifts is not to be explained by any difference in texture or topography, these being alike on both drifts, but is to be attributed to the difference in age. Throughout the first 3 feet in the forest fields leaching had been equally severe on both drifts. Even on the upland prairie the difference, in so far as most crop plants are concerned, is very slight.

In the case of the lowland prairie fields the drifts are quite similar, the third foot being neutral in all cases while the reaction of the upper sections varies much from field to field. In one field on each drift much carbonate was found at all levels. The nearness of the water-table to the surface on this type on both drifts has generally so checked leaching as to obscure any influence of age that might otherwise have been shown.

Depth of leaching of carbonates

It is evident from the above that if a characteristic difference in the extent of leaching is to be found in the case of the two areas compared the depth of examination must exceed 3 feet. On the Wisconsin till in Iowa Alden and Leighton (1, p. 88) found the depth of leaching to extend only to 2 to 3 feet from the surface, and in many places limestone pebbles were found at or near the surface. In contrast with this, on the Iowan they found the average depth of leaching to be from 4 to 6 feet,² with limestone pebbles mostly absent from the leached layer, they averaging only 3 per cent of the total number of pebbles present (1, p. 81 and 184). In the examination of 165 exposures in cuts and 250 auger borings they found less than one-tenth to show effervescence at a depth of less than 3 feet (1, p. 82).

Our five upland prairie and five forest fields on the older drift, in which 200 borings were made in securing samples, were found leached beyond the 3-foot level, and so do not differ in this respect from Alden and Leighton's description of the Iowan. On our five forest fields on the young drift, in which 100 borings were made, the leaching throughout the 3-foot section appears as complete as on the older drift. Even on the upland prairie on the latter drift two of the fields (I and II in table 21) showed almost as little evidence of carbonate, but the third foot in two was very calcareous and the second foot sufficiently so to indicate that on each of these some of the 20 borings had penetrated the unleached layer. Thus the leaching of the older and younger drifts that

² This depth of 4 to 6 feet generally includes 1 to 2.5 feet of leached soil and loess-like clay overlying 3 to 3.5 feet of leached till.

TABLE 23
Depth of leaching of carbonates

	OLD DRIFT					YOUNG DRIFT				
	Field I	Field II	Field III	Field IV	Field V	Field I	Field Ia	Field III	Field IV	Field V
Forest										
Depth of leaching (feet)	4.5	4.5	4.0	5.5	3.8	4.5	4.0	3.6	3.8	5.0+
Calcareousness of third foot*	Slight acidity	Very slight acidity	Medium acidity	Slight acidity	Strong acidity	Medium acidity	Slight acidity	Medium acidity	Very slight acidity	Slight acidity
Upland prairie										
Depth of leaching (feet)	3.6	4.8	4.3	3.7	3.6	4.5	2.7	2.5	4.5	2.9
Calcareousness of third foot*	Slight acidity	Medium acidity	Medium acidity	Strong acidity	Medium acidity	Very slight acidity	3.55 per cent carbonate	7.12 per cent carbonate	Very slight acidity	1.23 per cent carbonate

* From table 21.

we have found in Rice County is similar qualitatively but not quantitatively to what Alden and Leighton report for Iowa. In this connection it should be emphasized that these writers were contrasting the Des Moines lobe as a whole with the older drift to the east, while we are intentionally confining our comparisons to a part of the extreme western edge of the Iowan with the adjacent and almost extreme eastern edge of the Wisconsin. Their reports do not indicate that they have sought to ascertain whether distinct differences in depth of leaching might be found upon the Des Moines lobe itself when one part is compared with another, as for example the eastern with the central or western.

If at the time of our collection of samples we had had before us the results of Alden and Leighton's studies we would have carried each of the borings down to the unleached till, but at that time our purpose was not to make any more exhaustive comparison of the carbonates than of various other important constituents. However, we have revisited the area very recently—since the advent of the winter—for a hasty exploration for the depth of leaching, making a boring or two in each of the twenty upland fields, as well as in many of those intervening, and examining the exposures in recent road cuts. Table 23 shows the depths at which effervescence was found in the various fields, with the use of cold dilute hydrochloric acid.

The upland prairie fields on the Wisconsin showed an average of 3.4 feet and on the older drift of 4.0 feet. The forest showed an average of 4.0 feet on the former if we omit field V, and of 4.5 feet on the latter. In that field we found a layer of sand and gravel at a depth of about 5 feet to underlie all the portion sampled, and down to this coarse layer no sign of carbonates was found.

DIFFERENCES IN COMPOSITION ACCORDING TO SOIL TYPE

The lowland prairie fields are the finest in texture and highest in nitrogen, organic carbon, phosphoric acid, magnesia and lime (both total and non-carbonate). The upland prairie occupies an intermediate position in regard to texture, content of nitrogen, and organic carbon. The forest is coarsest in texture and slightly the highest in silica and lowest in alumina. In potash and soda there is no distinct difference.

RELATION OF COMPOSITION TO DEPTH OF SECTION

The relations found to exist among the successive levels are not unusual. With increase in depth there is in general a decrease in the fineness of texture, as expressed by the moisture equivalent, but in more than half of the upland prairies no distinct difference was found between the upper and lower sections of the surface foot. In the forest fields the second foot shows a moisture equivalent equal to that of the overlying 6-inch layer, and on part of the lowland prairies there is little difference between the second and third foot-sections.

With an increase in depth nitrogen and organic carbon decrease rapidly, and phosphoric acid to a less extent, while carbonates if present at all, tend to increase. Where carbonates are absent from the 3-foot profile the degree of acidity is not distinctly related to the depth. Compared on the basis of the carbonate-free non-volatile portion of the soil, iron is found to increase with depth and magnesia to a less marked extent, while silica, alumina, titanium and potash remain practically constant. Soda is a little the highest in the surface section and non-carbonate lime, on the prairie types, tends to decrease with depth.

RELATION OF COMPOSITION TO AGE OF DRIFT

From the above detailed comparison of the properties and composition it is evident that to a depth of 3 feet the two drifts are very similar. Only in the content of carbonates, phosphoric acid, iron and titanium, as well as in the distribution of the gravel particles, do the differences appear of sufficient magnitude to merit further consideration in this discussion.

The first sign of age is to be sought in the extent of leaching of carbonates, in which the differences found are a distinct disappointment. On the younger drift the depth in the forest type averages about 4.0 feet and on the upland prairie 3.4, compared with 4.5 in the forest and 4.0 in the prairie on the older.

Iron and titanium are slightly higher on the older drift. As the alumina is not correspondingly higher, or the silica, magnesia, etc. lower, we are not justified in attributing this difference to an enrichment of the iron through the leaching out of the other constituents. The occurrence of a larger proportion of iron minerals among the gravel particles of the older drift suggests a higher original iron content.

Phosphoric acid is characteristically higher in the upper layers of the older drift, but this is to be attributed to the upward translocation by plants rather than to a concentration through leaching, if we assume that the original till on both drifts was alike in this constituent.

No characteristic differences are found in silica, alumina, magnesia, non-carbonate lime, potash, soda, nitrogen or organic carbon.

The gravel shows only a slight difference; on the older drift there is a more marked difference between the third foot and the overlying sections, as though weathering had proceeded farther on this.

While what differences were found in the gravel and in the chemical composition of the fine earth agree with the assumption that the drift in the eastern part of Rice County is the older, they are far too small to have suggested a difference in age, and they might be regarded as so slight as even to cast doubt upon the correctness of the line which Leverett has traced in Rice County as the boundary between the drifts, and hence upon the location of our western fields on the younger drift, were the boundary of the Late Wisconsin not so distinctly marked by the characteristic terminal moraine (fig. 3.)

Our results are not in accord with those found by Brown in comparing the soils on the Iowan and Late Wisconsin glaciations in Iowa and by Hopkins and Pettit in their earlier and more extensive studies in Illinois, in which they dealt with the Illinoian, Early and Late Wisconsin and possibly also with the Iowan.

Hopkins and Pettit (8), using samples from three levels, 1-6, 7-20, 21-40 inches, found a decrease in potash, phosphoric acid, and nitrogen to be associated with an increase in the age of the drift upon which the soils occur, the potash showing the most striking differences. Brown (3), using similar sections found the soils on the Late Wisconsin richer in potash, phosphoric acid, total lime, nitrogen and organic carbon than those on the Iowan.

We purposely selected fields so close to the eastern boundary of the younger drift in order to avoid differences in soil composition that might have resulted from differences in climate, in vegetation, or in both, as these would be liable completely to mask any differences in composition due to difference in age, if the sampled areas are very far apart. If we had compared samples taken from areas much farther removed from one another we would have found much greater differences in some constituents, as is evident from studies by P. R. McMiller and P. M. Harmer, which are to appear in later numbers of this series, but these differences we attribute to other causes than the greater age of the drift east of the Des Moines Lobe.

SUMMARY

1. A comparison was made of the composition and properties of the soils developed on neighboring areas of two drift sheets in southeastern Minnesota—the Des Moines Lobe of the late Wisconsin and the older glaciation exposed just to the east of this and referred to as the Iowan. The original till of these appears to have been very similar chemically and physically, and the areas compared are so near one another that they must have been subjected to the same climatic influences since the recession of the last ice sheet.

2. On each drift 15 virgin fields were sampled—5 on each of three soil types, viz., Carrington loam, naturally covered with deciduous forest, and Carrington silt loam and Fargo silt loam, both naturally in prairie. The samples were taken to a depth of 3 feet, in four sections—1-6, 7-12, 13-24 and 25-36 inches, 20 borings in each field.

3. Comparing the three soil types the Carrington loam (forest) was found coarsest in texture and slightly the highest in silica and lowest in alumina, the Fargo silt loam (lowland prairie) the finest in texture and highest in nitrogen, organic carbon, phosphoric acid, magnesia and lime, while the Carrington silt loam (upland prairie) occupies an intermediate position in texture and content of nitrogen and organic carbon. In potash and soda there is no distinct difference.

4. Comparing the four successive levels an increase in depth is found to be accompanied generally by an increase in coarseness of texture, regularly

by a decrease in nitrogen and organic carbon and less regularly by a decrease in phosphoric acid. In fields where carbonates were encountered they increased with depth, but where they were absent throughout the 3-foot section the degree of acidity is not distinctly related to the depth. When comparisons are made on the basis of the carbonate-free, non-volatile portion of the soil, with increase in depth iron and magnesia are found to increase slightly, non-carbonate lime to decrease slightly, and silica, alumina and potash to remain practically constant. The leaching effect upon the mineral constituents originally present in the drift has been practically confined to the carbonates.

5. Comparing the soils from the two drifts, type by type and level by level, no distinct differences are found in texture, or content of silica, alumina, potash, soda, magnesia, non-carbonate lime, nitrogen, and organic carbon. The soils on the older drift are considerably richer in phosphoric acid, especially in the upper levels, and contain appreciably more iron and titanium. The carbonates have been so thoroughly leached from the whole 3-foot section in the forest fields on both drifts that a distinctly acid reaction is found at all levels. The same holds true of the upland prairie on the older drift, but on part of the fields on the younger drift carbonates are found in the third and even in the second foot. A later exploration of the levels below the third foot shows that on both the upland soils types the depth of leaching out of carbonates is in general somewhat greater on the older drift.

6. It would appear that the only distinct influence that the greater age of the Iowan drift has been able to exert upon the soils developed upon it is confined to the leaching out of carbonates to a greater depth and possibly an enrichment of the surface layers in phosphoric acid through the agency of plants.

7. It appears that in a comparison of the chemical composition of the soils on drift sheets neither of which is older than the Iowan the only distinct difference that may justly be attributed to the difference in age is confined to the relative depth of leaching of carbonates. The greater concentration of phosphate near the surface on the older drift, observed in the areas compared in this study, may not be found elsewhere.

8. It appears probable that the marked differences in content of nitrogen and organic carbon reported from comparisons of soils on Wisconsin and Iowan drifts are due to differences in the vegetation and climate that have been associated with the particular portions of the two sheets employed in the comparison.

9. Both the Iowan and Wisconsin glaciations appear to have been too recent to allow sufficient time for any appreciable leaching out of the original content of potash, of non-carbonate lime and magnesia, or of silica, at least in the case of the till or of the finer-textured assorted material from this.

10. The question is also raised as to whether there is any satisfactory evidence of the leaching out of the four constituents just mentioned, even in the case of the oldest drift sheets.

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PLATE 1

ILLUSTRATIONS SHOWING THE CHARACTER OF VEGETATION ON THE FOREST TYPE

FIG. 1. Field V on the Old Drift; the samples were collected on the portion with the trees still standing.

FIG. 2. In the midst of Field IV on the Young Drift, showing the cover of leaves on the forest floor.



FIG. 1



FIG. 2

PLATE 2

ILLUSTRATIONS SHOWING THE CHARACTER OF THE ONLY REMAINING UNPLOWED TRACTS ON
UPLAND PRAIRIE

FIG. 1. Field V on the Young Drift consists of the long unplowed strip in the fence line between farms.

FIG. 2. Roadside strip on the Young Drift, Field II.



FIG. 1



FIG. 2

PLATE 3

ILLUSTRATIONS SHOWING THE TOPOGRAPHY OF THE LOWLAND PRAIRIE FIELDS

FIG. 1. Field III on the Old Drift, the broad roadside strip at the left.

FIG. 2. Field II on the Young Drift. It lies at the foot of the slope and to the right of the fence.



FIG. 1



FIG. 2

A PITLESS LYSIMETER EQUIPMENT

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The use of outdoor lysimeters offers an attractive field for research in soil chemistry. The cost of installation, however, is a deterrent to a more general utilization of this type of equipment. It is necessary to afford protection to the receivers provided for the collection of the leachings and to insure against undesirable temperature ranges. If the equipment is extensive, it is also desirable to provide for the comfort and efficiency of the persons engaged in weighing, or measuring, and sampling the percolations. The expense of constructing a substantial concrete cellar or terrace walls is one of the principal factors in the cost of such an installation. At the present time there are in operation at the University of Tennessee Agricultural Experiment Station, three distinct types of concrete structures devoted to studies upon the leachings from soils subjected to experimental treatment.

The first and second installations have been described in two papers by the writers of this article (1, 2). The third installation has not been described. Recently, still another equipment has been installed. This last equipment is distinctive, in that no surface or sub-surface concrete enclosure is required. A description and illustrations of it are here offered, with the hope of stimulating research along lysimeter lines by pointing out that the tanks of this type can be installed at relatively low cost. Even under the prevailing abnormal conditions a 12-unit system has been installed at a total cost of \$500; while a 34-tank system involving tanks of greater area and less depth has been contracted for at a total cost of about \$1400. As illustrated in plate 1 and figure 1, each lysimeter is composed of a pair of heavy (14-gauge) cylindrical galvanized "ingot iron" tanks. The soil-container is 6 feet 3 inches long, with an inside diameter of 12 inches. The bottom of this tank is slightly conical in shape, with a flexible block-tin tube outlet. Around the outside of the top is riveted a $\frac{1}{4}$ by 1-inch iron band. Soldered to the outside of the tank and just under this band is a 2-inch copper rim, or water shed, as a protection against movement of rainfall down the outside of the tank. Twisted iron handles, or loops, also are fastened to the inside of the inner tank at the top, in order to facilitate lifting when desired. The inside soil-container tank slides into the outer tank which is 7 feet and 3 inches deep. On the inside of the outer tank are riveted three iron lugs, or supports, upon the upper ends of which the inside tanks rest, the bottom of the inside tank

being 1 foot from the bottom of the outside tank. The percolates from the inside tank are caught in this reservoir of 1 foot depth and slightly greater diameter.

The bottom of the outside tank is on the bevel, though the sides of the tank extend below to a horizontal plane and rest upon a 6-inch concrete base 15 inches square. This concrete base prevents settling of the sharp edges and thus insures against any disrupting strain upon the bottom of the outside tank. A hole is drilled in the side of the outer tank at the lowest point of the beveled bottom for insertion of a block-tin L which is heavily soldered to the tank wall and to

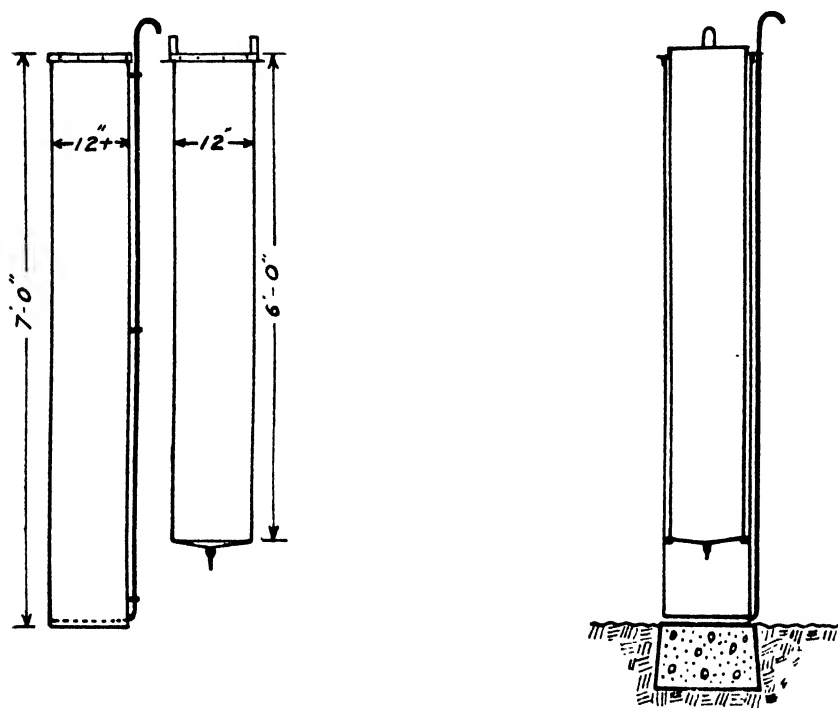


FIG. 1. SKETCH SHOWING INTERIOR ADJUSTMENTS FOR INSERTION OF THE SOIL CONTAINER TANK IN THE OUTSIDE RESERVOIR TANK AND CONCRETE BASE

the end of the perpendicular $\frac{1}{4}$ -inch galvanized iron pipe which extends several inches above the ground level. To the top of this perpendicular tube, which is attached to the exterior side of the outside tank, a block-tin goose-neck is soldered. A slit is provided in the copper rim to permit passage of the $\frac{1}{4}$ -inch upright galvanized iron pipe. The leachings, which pass down through the inner tank to the outer tank reservoir are pumped up this upright goose-necked pipe into individual asphaltum-coated galvanized-iron containers, one of which is shown attached to the tank and pump in plate 2. These leaching-containers have convex tops tightly soldered around the edges and two necks, one for in-flow of leachings and one for suction outlet to the foot pump. The

tops of the containers are provided with 6-inch crimped lids which can be made airtight by means of a vaseline-paraffin mixture seal; and which also afford means of ready access for examining, painting, or coating the interiors.

In placing the soil contents, a perforated disc or asphaltum-coated wire cloth strainer is placed over the central outlet of the inner tank and a thin layer of coarse sand introduced. As definite amounts of soil are added, careful and uniform tamping should be insured by means of a circular tamp of slightly less diameter than the tank. Should a periodic study of the different zones of the soil column be contemplated, a $\frac{1}{16}$ or $\frac{1}{8}$ -inch mesh asphaltum-coated wire-cloth disc may be inserted at the desired depths. This insures definite demarcation of the prescribed zones at the time of dismantling. By proper duplication, this type of equipment adapts itself to a continuous study of the periodic and progressive residual effects induced by particular treatments, according to indications afforded by the leaching data. When it is desired to make such residual studies upon the different zones, a steel bar is placed through the iron-loop handles of the inner tanks and they are hoisted and removed by means of the block and tackle and tripod device shown in plate 2 (fig. 2). The soil-filled tanks are then placed in a horizontal position to facilitate removal of soil and subsoil zones. The tanks are then available for new or repetition studies.

While the illustrated tanks are all of 12-inch diameter and 6 and 7-foot depths, the principle may be adapted as applying to larger diameters and lesser depths. As previously stated, an additional set of this character are being placed shortly, upon bases already provided.

Local conditions, as to periodicity and volume of rainfall and depth of frost line, of course, are to be considered in the use of this type of equipment. In those climates where freezing below the 1-foot line over extended periods would be anticipated, there would be probably a minimum of winter leachings, which would mean little, if any, required attention or discomfiture during the intensely cold weather. On the other hand, in moderate climates where the soil seldom freezes for any length of time and where winter rains result in leachings, the short period of exposure experienced while pumping the leachings for conveyance to the laboratory would not constitute a serious objection. While the volumes of the leachings are not to be observed by the eye, nevertheless, knowing the relationship between rainfall and leachings, the record of precipitation will serve as an adequate guide relative to the frequency of removal of leachings.

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PLATE 1

FIG. 1. Outside tank and inside soil-container tank.

FIG. 2. Outside tank with inside tank in place.

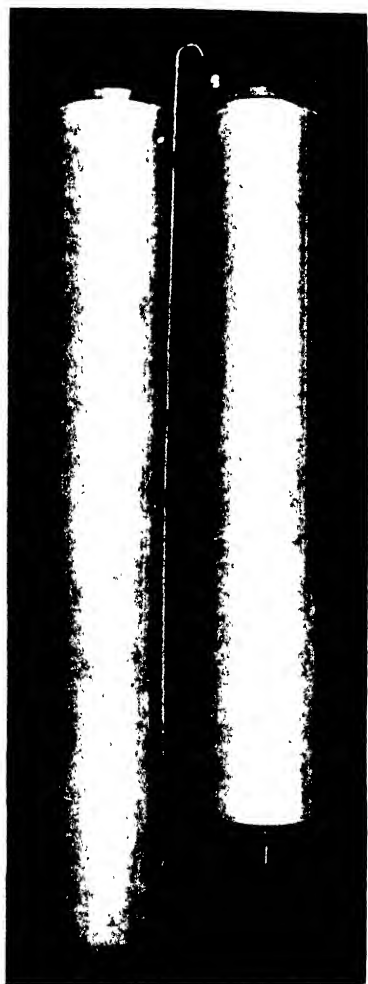


FIG. 1

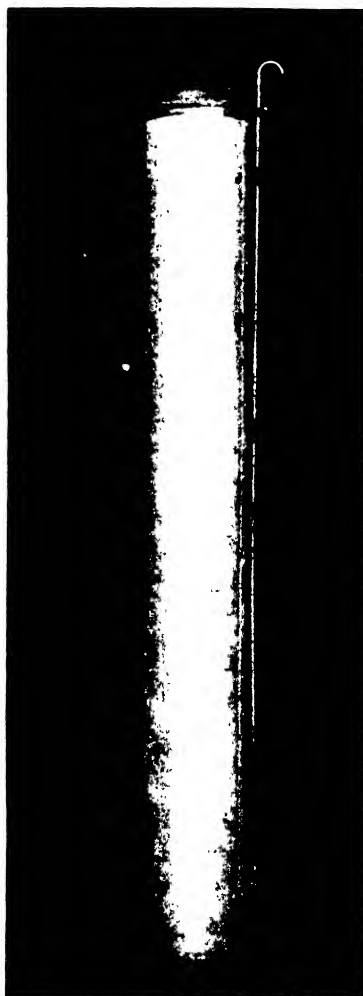


FIG. 2

PLATE 2

FIG. 1. Illustration showing the arrangement of the tanks after placing the inside tanks and rims masking the outside tanks, and the apparatus for pumping up the leachings.

FIG. 2. Tripod and tackle and pulley device for removing the soil-container inside the tank when dismantling for periodic studies.



FIG. 1

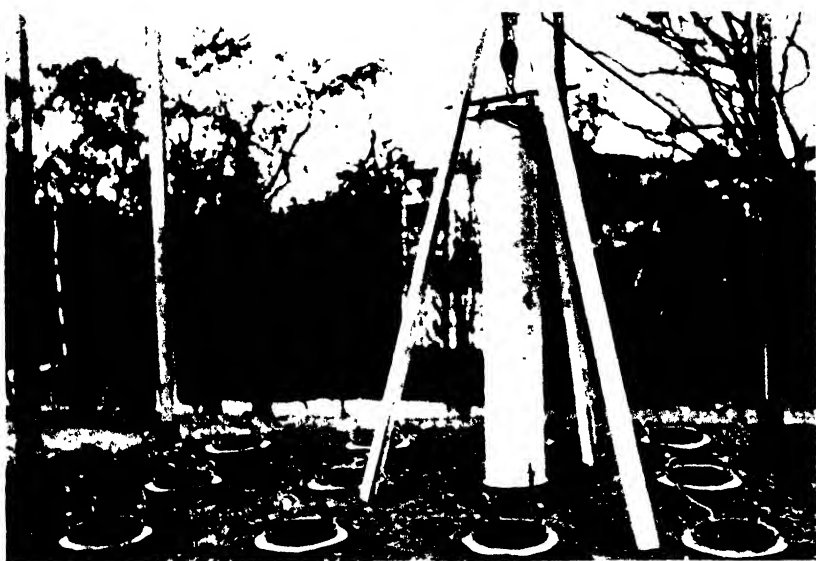


FIG. 2

THE MOVEMENT OF SOIL MOISTURE

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I. INTRODUCTION

The quantity and diversity of the experimental work that has been done on soil moisture attests the importance of the subject. The motive, as with other phases of agricultural research, has been primarily economic in character and for that reason little attention has been given to the theoretical aspect of the problem, although a number of excellent articles have been written from this point of view. As experimental facts accumulate, however, the need for a generalization increases. With this thought in mind and with the hope that those who are interested will offer helpful suggestions, the authors have been led to prepare this short article as an introduction to the subject.

The experimental curves presented below are not wholly conclusive and it is possible that certain modifications in the original hypotheses may be found necessary, but the general conformity in theory and experiment would seem to lend a considerable amount of evidence to the approximate validity of the assumptions that have been made.

The first section is merely an attempt to outline in a language more generally understood the trend of thought embodied in the mathematical development of the second part. The differential equation (4) is the direct consequence of equation (1), which contains the assumptions which have been made, and equation (2), which is a well known equation in hydrodynamics, the validity of which in this case will not be questioned. To those who are unfamiliar with the methods of the calculus an equation such as (4) does not convey an intelligent idea, and the methods which are resorted to in applying it to experimental facts will likewise be somewhat vague. It is hoped, however, that some satisfaction may yet remain by passing over this part of the discussion, and judging of its fruitfulness by a comparison of the graphical representation of its predictions with the graphical experimental facts.

It should be pointed out that the trend of natural phenomena may be influenced by numerous agencies or factors and an attempt to introduce them all

¹The formal development of the mathematical material of this paper was made by the senior author as an outgrowth of the earlier work of the junior author, who has assisted in clarifying this material for publication

explicitly in a mathematical formula purporting to describe such phenomena would be foredoomed to failure. If, however, minor factors may be temporarily ignored and the major ones divided into a small number of groups, it may thus be possible to construct in the imagination an ideal problem which in reality may differ only slightly from the actual case but the solution of which may lie within our power.

Let us, therefore, regard the soil as a rigid, homogeneous configuration of heterogeneous individual particles which does not change with the time, but through which the moisture moves; and we shall attempt to specify the density of the moisture in the soil, i.e., the aggregate amount in unit volume of soil, in terms of space coördinates and time, together with constants which characterize the soil and soil solution. This, in fact, has been the primary object of numerous investigations.

If we can by the methods of mechanics arrive at a solution of this problem for the ideal case, we shall thus provide an avenue leading to an approximate solution of the more difficult problem offered by actual soils whose individual particles are not fixed in position and whose soil solution is not a simple substance.

As is customary and essential in the analytical treatment of phenomena depending upon quantities which vary with space and time, we seek to specify the facts with respect to an element so small that average conditions from point to point within the element are sufficiently alike that the differences may be neglected. In the present instance we may therefore fix our attention upon a small cubical element of the liquid (fig. 1) at any selected region of the soil space, and, if we successfully describe its position and its motion in terms of known quantities and known laws in a comparatively simple way, well known methods of mathematics will in all probability point the way to a final solution.

The forces acting upon such an element of liquid may be divided into two kinds, viz., those which are independent of adjacent surrounding liquid and those which are due to the presence of adjacent liquid particles. The only force of the first type is the force of gravity, which is constant in direction and magnitude and is proportional to the mass of the element. Of the latter there are three, viz., pressure, cohesion, and friction, the last-named depending upon the relative velocity of the element. Cohesion may be regarded as a negative pressure (or tension) and if we so regard it there will remain but two forces of the second kind. We may therefore say that for the chosen element there exists a force acting vertically downward proportional to the mass, a pressure on each of the six sides, and a frictional drag due to the relative slipping of the element, which may be zero for any or all of the six sides, depending upon the relative velocity at each side. As stated, the force of gravity is constant for each unit of mass of the liquid regardless of time and position. Each of the others, however, will differ in general from point to point in the liquid and at each point may change with the time, so that the

specification of each will require, in addition to the characteristic constants of the soil and liquid, coördinates of space and time.

It should be noted that this method of describing the acting forces in no way depends upon the particular location of the element of volume and is therefore a general statement which applies to each of the various elements throughout the entire region with which we are concerned. There remains, then, the real problem of specifying just how these forces depend upon space and time, or, in other words, the determination of the functional relation between such forces and the coördinates mentioned.

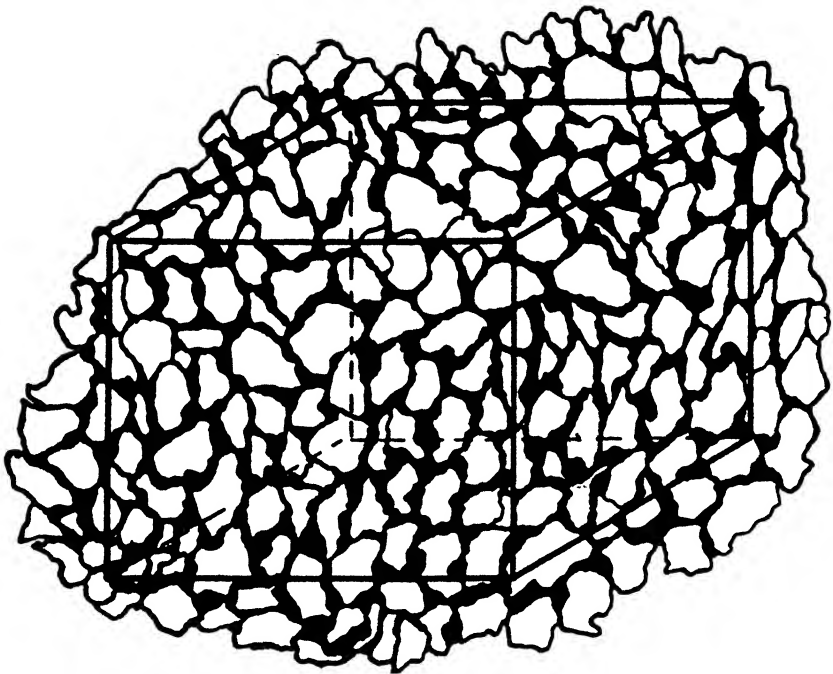


FIG. 1. DIAGRAM OF CUBICAL ELEMENT OF SOIL

Let us locate the volume element by specifying its distances, x , y and z , measured from each of three perpendicular intersecting planes fixed in the soil. As stated, each such element should be sufficiently small that the conditions of pressure, moisture density, etc., are practically constant throughout the element but sufficiently large to include a representative mass of soil aggregate. It should be borne in mind that within the element discontinuities will arise as we pass from point to point, and in making use of this method of analysis it must be remembered that average conditions particularly concern us. The average pressure, whether it arises from the weight of superimposed liquid or from the pressure of a near-by curved surface, will in general be different for the various elements.

For bodies moving in response to conservative forces the resultant of the effective forces is a measure of their acceleration in the direction of the resultant. Where friction comes into play, however, this is not true but a limiting velocity is soon reached when the frictional force becomes equal and opposite to the resultant of the impressed forces. For small velocities of such magnitude as will be encountered in the soil, this frictional force is directly proportional to the velocity. If, for example, water is forced through a small pipe of regular or irregular section, including the pores of a homogeneous soil, the mean velocity is found from theory (8) and experiment (9) to vary directly as the pressure gradient² (6) with a proportionality factor which involves the shape and size of the tube. Where the soil is unsaturated and movement takes place in response to capillary forces, it is evident that the degree of saturation may become an additional factor, but in the absence of direct evidence that this has an appreciable effect upon the inherent moisture conductivity, we may *temporarily* ignore the moisture concentration.

The statement of this hypothesis in mathematical language will furnish a starting place for the development of the problem. Since, as stated, the force of gravity must be taken into account as well as the pressure, it becomes necessary to include its effect in the equation. The potential gradient is a force which is proportional to the mass, and, as may readily be shown, the pressure gradient is dimensionally a force per unit volume, and in order to bring these two forces together a potential function Φ , depending upon the coördinates x , y , z and t , has been introduced as a measure of the potential energy per unit mass of the liquid. We may think of this as the sum of three independent terms, viz., π , ψ , and φ , the first being the energy due to hydrostatic pressure, the second the energy due to capillary pressure, and the third the energy due to gravity. Since the negative gradient of this potential becomes a force per unit mass, it is evident that the density ρ must be introduced as a factor, and in this way we arrive at equation (1), as may be readily shown.

Without attempting to become familiar with the meaning of the mathematical symbolism involved, the reader should note that this equation is a statement of the hypothesis, viz., that the mean velocity of any given portion of liquid through the soil is proportional to the pressure gradient, or in equivalent language, to the product of the amount of moisture in unit volume at the point in question and the rate of change of the potential energy per unit mass characteristic of such point as we move from point to point in the given region.

It should be pointed out here again also, that while the velocity of the element of liquid may ultimately depend upon a large number of factors such as

² For exceptions the experimental work of King as cited below is of interest.

By the term *gradient* as applied to any such quantity as pressure, potential, temperature, density, etc., we mean the magnitude of the change of such quantity as we move from point to point in the direction of greatest change.

the surface tension of the liquid, the coefficient of viscosity of the liquid, the porosity of the soil, the size and shape of the individual grains, the temperature, the barometric pressure, and so on, yet the effect of each will be made manifest through one or the other of the three quantities, K , ρ , and Φ .

It is evident that whatever may be the character of the motion, the rate of accumulation (positive or negative) of liquid within a given region is determined uniquely by the velocities of the numerous individual particles located on the boundary of such region, or, in other words, the increase or decrease of moisture must be accounted for by the flow through the boundary of such region. The mathematical expression of this fact is in the form of another equation (2), the equation of continuity. And, if we multiply equation (1) by the moisture density ρ and substitute in equation (2) we obtain finally equation (3), or, the equivalent form, equation (4), a relation which expresses quantitatively and briefly our hypothesis combined with the equation of continuity.

Furthermore, since the density of moisture at any point depends upon the original density, the rate of accumulation, and the time, it would seem only logical to expect that a knowledge of these quantities and a knowledge of limiting conditions, together with methods of calculation, should be sufficient for the determination of the moisture density at any given region at any given time, which is the end sought in the investigation. The application of the equation to experimental facts is therefore very largely a matter of mathematical technique and, as previously stated, it is not expected that the non-mathematical reader will be able to follow the steps in detail. In the mathematical development, however, a number of simple expressions have been deduced as a result of the application of the general equation to certain kinds of soil-moisture phenomena, and in some cases experimental results are shown in graphical form, indicating in general the confidence that may be attached to the validity of our hypotheses.

In equation (4) there appears a factor p , the capillary pressure, which we are unable to measure directly, and an attempt has been made to evaluate this in terms of the moisture density. In a previous article (1) one of the authors has made use of the tentative relation,

$$\frac{\partial p}{\partial x} = \frac{k_2}{\rho^{\frac{4}{3}}} \frac{\partial \rho}{\partial x} \text{ or } \nabla p = \frac{k_2}{\rho^{\frac{4}{3}}} \nabla \rho \quad (a)$$

and in a subsequent article (4) it has been pointed out that there is some experimental evidence indicating that the capillary potential³ ψ is a linear function of the reciprocal of the moisture density ρ over a considerable range for a large number of soils, thus:

$$\Psi = \frac{c}{\rho} + b \quad (b)$$

³ The symbol φ has been used previously (4) for this potential. It is perhaps better, however, to reserve φ for the gravitational potential.

and, since, as previously stated, the potential gradient and the pressure gradient differ by a factor of the dimensions of ρ , it follows that the relation must be of the form,

$$\nabla p = \frac{c}{\rho} \nabla \rho \quad (c)^4$$

where c is a constant satisfying the relation (b). We make use of equation (c), however, subject to any modification that may be found necessary in equation (b).

The constants in the equations which have been plotted in the second section were determined arbitrarily. They involve not only the boundary conditions but also constants which are characteristic of the soil and soil solution. It is not our purpose in this brief discussion to consider in detail the magnitude and significance of these characteristic constants. In the notation, however, an attempt has been made to preserve the identity of Slichter's transmission constant, designated by K ; the slope parameter of the Briggs potential function, designated by c ; the capillary transmission constant k ; and the lenticapillarity constant designated by the small Greek letter κ .

II. MATHEMATICAL DEVELOPMENT OF THE PROBLEM TOGETHER WITH EXPERIMENTAL DATA IN GRAPHICAL FORM

As stated in the introduction, we make the original assumption that the mean velocity of the water through the soil is proportional to the pressure gradient, or more generally, to the force per unit volume, and we therefore write,

$$v = K\rho\nabla\Phi \quad (1)$$

where

- v = mean velocity at a point in the soil
- K = a proportionality constant
- ρ = moisture density at the point
- Φ = sum of three potentials, π , Ψ and φ
- π = potential due to hydrostatic pressure
- Ψ = potential due to capillary pressure
- φ = potential due to gravity

⁴ Since this article was submitted for publication some unpublished experimental work of Mr. Thomas of the Agronomy Department has come to our attention indicating that the vapor pressure is approximately an hyperbolic function of the moisture content. By a well known method it may be shown that the capillary pressure p is related to the vapor pressure π as follows: $\pi = p - RT \log \frac{p}{p_0}$ and a simple algebraic substitution leads to a converging series which for finite values of the vapor pressure, differing only slightly from the average vapor pressure of water, is identical in form with equation (b).

$$\nabla = \frac{\partial}{\partial x} + \frac{\partial}{\partial y} + \frac{\partial}{\partial z} \quad (\text{a mathematical operator})$$

$$\nabla \Phi = \frac{1}{\rho} \nabla (P + p) + \nabla \varphi$$

P = hydrostatic pressure

p = capillary pressure

The equation of continuity must be everywhere satisfied, and we therefore write,

$$\frac{\partial \rho}{\partial t} = - \nabla (\rho v) \quad (2)$$

Multiplying (1) by ρ and substituting in (2), we obtain,

$$\frac{\partial \rho}{\partial t} = - K \nabla (\rho^2 \nabla \Phi) \quad (3)$$

or the equivalent form (remembering that $\nabla^2 \varphi = 0$)

$$\frac{\partial \rho}{\partial t} = - K \{ \rho \nabla^2 (P + p) + \nabla \rho [\nabla (P + p) + 2 \rho \Delta \varphi] \} \quad (4)$$

The product in the second member is scalar.

The following important cases may be considered in the solution of equation (4).

(1) *When P is finite, $p = 0$, $\nabla p = 0$, $\frac{\partial \rho}{\partial t} = 0$.* This case is realized when the pore space of the soil is full, and we have,

$$\nabla^2 P = 0 \quad (I)$$

Detailed consideration of this case has been given by Slichter (9) and will not be discussed here.

(2) *When p is finite, $P = 0$.* This is the most common condition met with in irrigation practice. For this case equation (4) reduces to,

$$\frac{\partial \rho}{\partial t} = - K \{ \rho \nabla^2 p + (\nabla p)^2 + 2 \rho \nabla \varphi \nabla \rho \} \quad (II)$$

For reasons discussed elsewhere in this article, we make the tentative assumption that

$$\Psi = \frac{c}{\rho} + b$$

which leads to

$$\nabla p = \frac{c}{\rho} \nabla \rho$$

and if we make this substitution for ∇p in (II), we obtain,

$$\frac{\partial \rho}{\partial t} = -Kc \left\{ \nabla^2 \rho + \frac{2\rho}{c} \nabla \varphi \nabla \rho \right\} \quad (\text{II}')$$

This becomes for a steady state,

$$\nabla^2 \rho + \frac{2\rho}{c} \nabla \varphi \nabla \rho = 0 \quad (\text{II}'')$$

Integrated for vertical flow and so choosing the coördinates that $\frac{\partial \rho}{\partial z}$ is negative, this becomes,

$$\rho = A \tan (B-Cz) \quad (\text{a})$$

where A , B , and C are constants involving the characteristic constants of the soil and soil solution, the gravitational acceleration constant g , together with the boundary conditions; and z is the distance measured vertically from an appropriate origin.

In the special case where $\rho = \rho_0$ when $z = 0$, and $\frac{\partial \rho}{\partial z} = 0$ when $z = \infty$, we obtain,

$$\rho \left(z - \frac{c}{\rho_0 g} \right) = - \frac{c}{g} \quad (\text{b})$$

In figure 2 are plotted experimental curves showing the distribution of moisture with height in a series of soil tubes of about 100 inches in height, which had been saturated with water and allowed to stand for a long time, various types of soil being represented. These curves were taken from data given by King (7). Superimposed are plotted with a broken line representative curves with arbitrary constants for families (a) and (b). King states that the sands continued to drip for a period of about two and a half years and it is possible that they had not attained a condition of equilibrium when the analyses were made. It is of interest to note also that one of the authors (10) found an equation of the family (b) satisfied approximately for Greenville soils in 24 to 48 hours after irrigation, this condition representing, however, a condition of steady motion, the moisture density diminishing with the depth. As pointed out in a previous publication (2) the moisture density ρ should be interpreted as that in excess of what has been designated as the lento-capillarity point. Another alternative would be to introduce this additional characteristic constant κ . Equation (b), for example, would thus become,

$$(\rho - \kappa) \left(z - \frac{c}{\rho_0 g} \right) = - \frac{c}{g} \quad (\text{b}')$$

The lento-capillarity parameter κ is a measure of the ordinates of the respective asymptotes of the curves.

(3) When p is finite, $P = 0$, and $\nabla\phi\nabla\rho = 0$. This case is realized where the water flows horizontally under the influence of capillary pressure. The product $\nabla\phi\nabla\rho$ vanishes in this case, since it is the scalar product of perpendicular vectors, and equation (4) reduces to,

$$\frac{\partial\rho}{\partial t} = -Kc\nabla^2\rho \quad (\text{III})$$

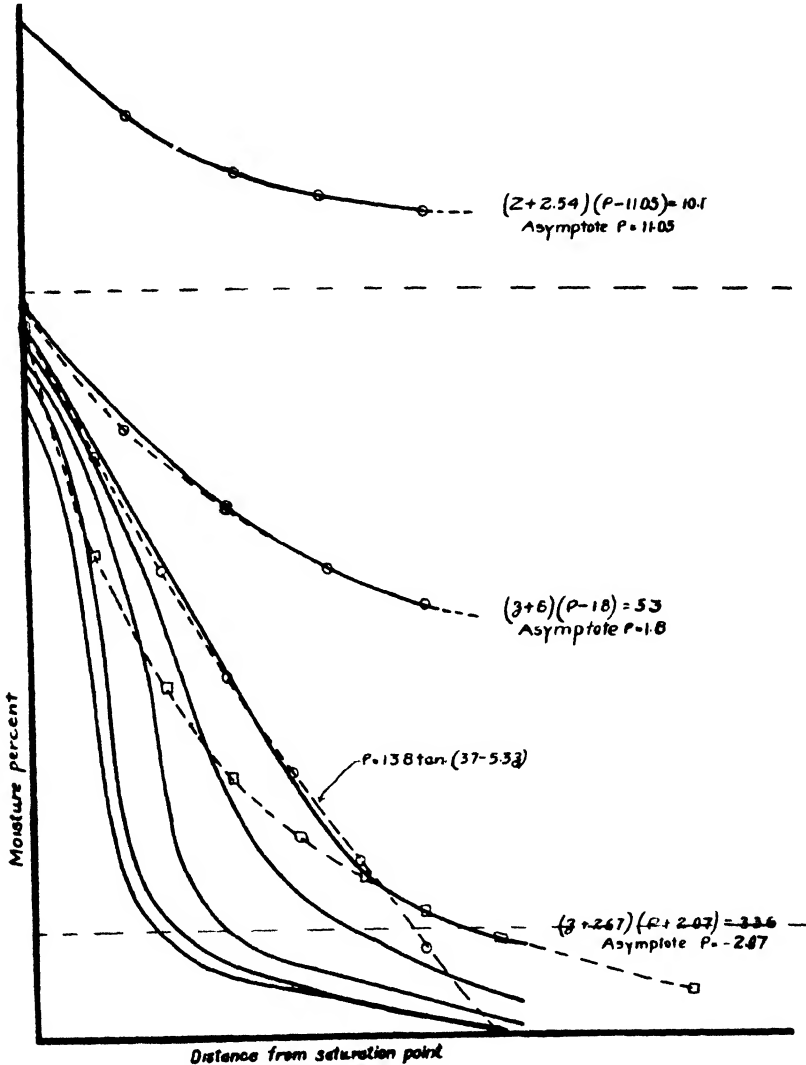


FIG. 2. THE DISTRIBUTION OF MOISTURE WITH DISTANCE FROM WET END (BOTTOM OF TUBE); PLOTTED FROM DATA GIVEN BY KING

This will be recognized as the well known heat equation, with a diffusion constant Kc ($= k$), i.e., the product of Slichter's transmission constant K and the slope parameter c in the Briggs potential equation. This will differ

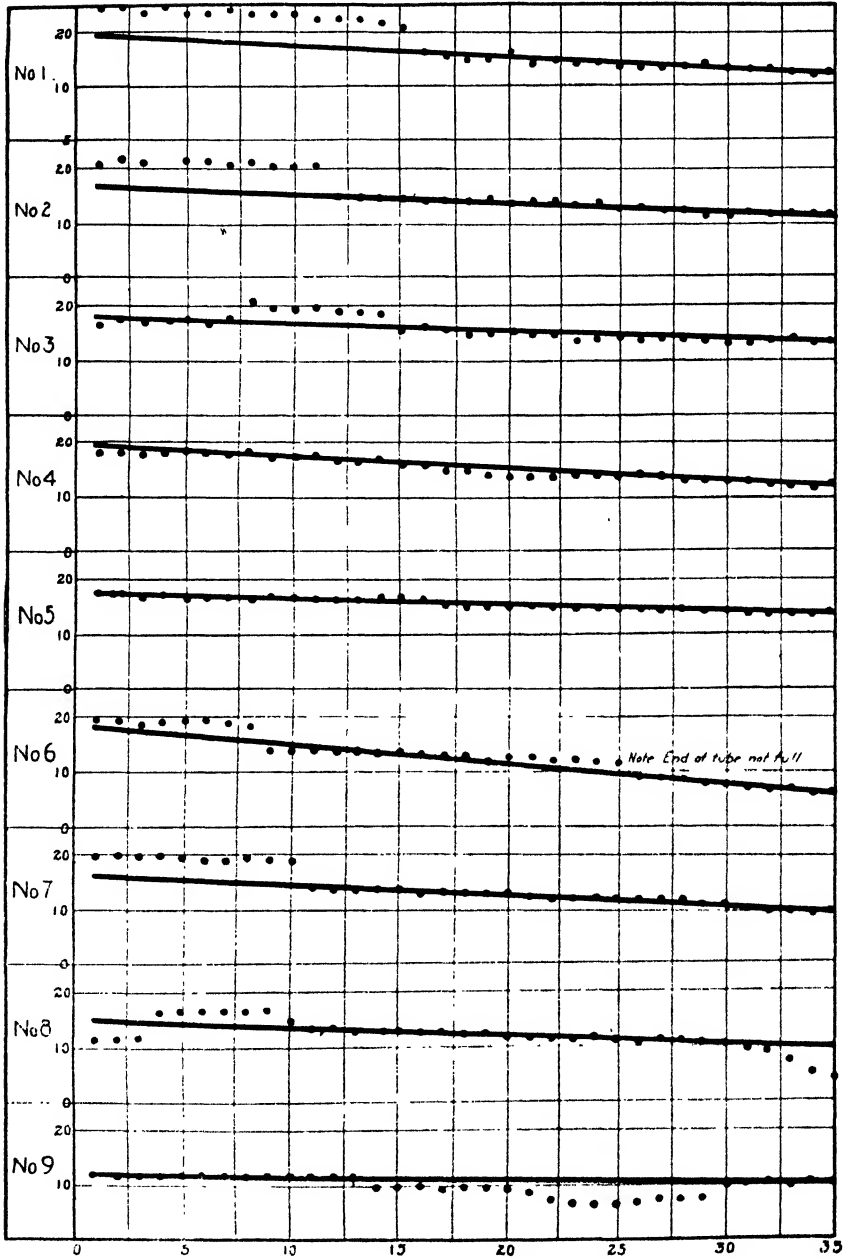


FIG. 3. THE DISTRIBUTION OF MOISTURE WITH DISTANCE FROM WET END FOR A SERIES OF HORIZONTAL TUBES AFTER THE MOISTURE HAD BEEN ALLOWED TO MOVE LATERALLY ABOUT 24 INCHES FROM ONE END WHICH WAS KEPT SATURATED

After reaching this point the source of supply was discontinued for about four months before analyses were made. The ordinate is given as per cent on the dry basis and the abscissa as inches from the wet end.

from the capillary transmission constant as previously defined and measured (3) by a factor ranging from about 1.3 to 1.7.

When solved for a steady state, we have,

$$\nabla^2 \rho = 0 \quad (\text{III}') \quad (a)^5$$

and for one dimensional flow,

$$\rho = Ax + B \quad (a)^5$$

In figure 3 are plotted nine curves representing the moisture distribution in a series of nine horizontal tubes 100 cm. long which had been filled with dry soil and irrigated by capillarity from one end. After a period of 7 days when the water had traversed about three-fourths the length of the tubes the source of water was discontinued. The tubes were allowed to stand for about 4 months and moisture determinations were made, one tube being analyzed each week for 9 weeks. These curves are shown here to illustrate the approximately linear distribution of the moisture in the tubes. It is evident, however,

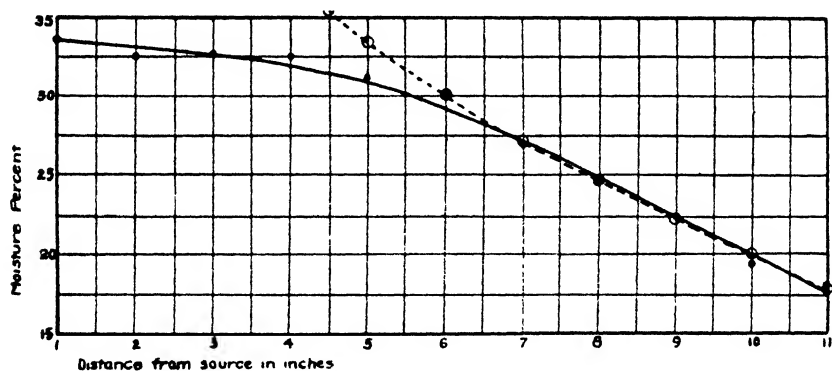


FIG. 4. DISTRIBUTION OF MOISTURE FOR APPROXIMATELY STEADY STATE FOR RADIAL FLOW

that an insufficient range of time and distance is represented completely to verify equation (a). Since also there was a slow readjustment in process the condition was only approximately steady.

For the case of radial flow in two dimensions, equation (III) becomes when integrated for a steady state,

$$\rho = C \log (x + 1) + \rho_0 \quad (b)$$

and in figure 4 are plotted the results of a preliminary test of the moisture distribution in the soil which had been irrigated from a central irrigation ditch, and the water allowed to move horizontally in all directions for a period of about 160 hours before moisture determinations were made. The soil was covered with a piece of cardboard to minimize the effect of evaporation. It can only be assumed, however, that this represents approximately a steady

⁵ If equation (a), p. 219, be used in place of (c) p. 220, this equation becomes $\rho = (Ax + B)^{\frac{1}{2}}$, as shown in a former publication (1).

condition. The theoretical curve is shown as in previous cases. It is to be expected that the theoretical curve will represent the moisture distribution only beyond the point of complete saturation.

(4) *When $p = 0$, $P = 0$, $\nabla\varphi = -g$, with no constraints except frictional resistance which is proportional to the length of the water column, which in turn is proportional to the mass.* This case would be realized were it possible to allow a column of water completely filling the pore space of the soil to drop under the influence of gravity without developing surface energy at either end of the column. In this case $\frac{\partial\rho}{\partial t} = 0$, ρ is constant, and all the terms in the right-hand member of equation (4) are zero. Equation (1), however, becomes,

$$v = K\rho\nabla\varphi = -K\rho g = \text{constant} \quad (\text{IV})$$

It is of course impossible fully to realize this case in practice (A, fig. 10), since $\nabla p = \frac{c}{\rho}\nabla\rho$ is finite at the water front, although it may become negligible in comparison with $\rho\nabla\varphi$ in a short time. If we consider the case of vertical flow, however, and write,

$$\begin{aligned} \rho v &= K\rho\nabla p + K\rho^2\nabla\varphi \\ &= Kc\nabla\rho + K\rho^2\nabla\varphi \\ &= -K\rho^2g + Kc\frac{\partial\rho}{\partial z} \quad (\text{since } \nabla\varphi = -g) \end{aligned}$$

and consider the value of ρv at the water front, we have

$$\rho_a \frac{\partial a}{\partial t} = -K\rho_a^2g + Kc\frac{\partial\rho}{\partial z}_{(z=a)}$$

and if we substitute

$$\rho = \rho_0 + \alpha e^{-\beta t}$$

(where ρ_0 and α are independent of t)

which may be made to satisfy (III), and (II) to a first approximation, we may write,

$$\frac{\partial\rho}{\partial z} = -Ae^{-\beta t}$$

if ρ_0 is also independent of z and $A\left(=\frac{\partial\alpha}{\partial z}\right)$ is independent of t . And from this we obtain,

$$\frac{\partial a}{\partial t} = -K\rho_a g + \frac{Kc}{\rho_a} Ae^{-\beta t}$$

and

$$a = c_1 t + c_2 (1 - e^{-\beta t}) \quad (\text{approximately}) \quad (\text{a})$$

As will be noted from the manner of performing the integration, a positive correction term should be added for small values of t .

In figure 5 are given in graphical form the results of an experiment performed in the laboratory illustrating this case. At the top of a column of sand originally air-dry about 3 meters high and 3.5 cm. in diameter, was fed a stream of water just sufficient to keep the surface covered completely, and the distance of the water front from the surface was recorded with the time and the curves plotted as shown. The lower curve represents a coarse sand of 0.06 cm. mean diameter, the next a medium sand of 0.04 cm. diameter, and the top a fine sand of 0.03 cm. diameter. The experimental points fell almost exactly on the curve and are not shown, but the theoretical points as calculated from equation (a) are shown with a circle.

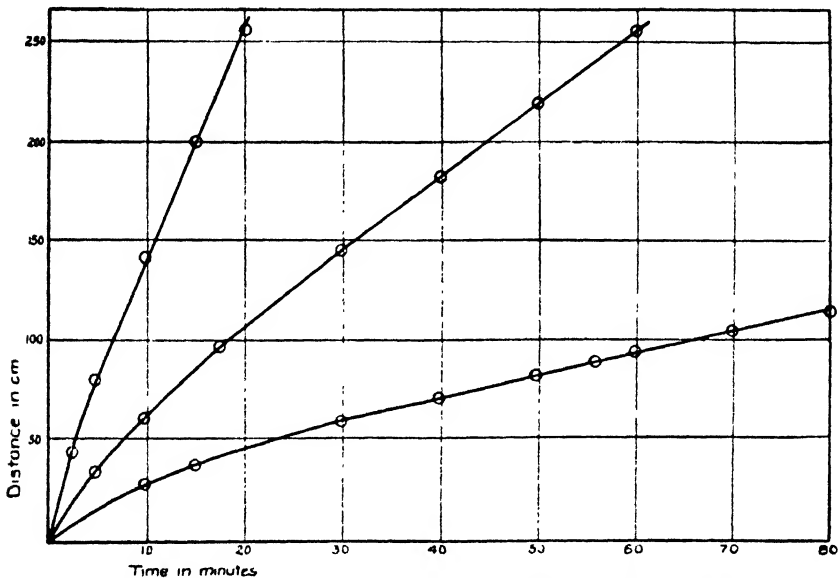


FIG. 5. THE DOWNWARD MOVEMENT OF A COLUMN OF WATER THROUGH SAND PARTICLES OF VARIOUS SIZE

The surface of the sand was kept saturated in an attempt to approximate complete saturation throughout the column from the source to the water front.

It is evident that the locus of (a) will range from a straight line to an exponential curve with asymptote c_2 , depending upon the ratio of c_1 to c_2 , this ratio increasing as the soil changes in texture from clays to sands. For horizontal capillary flow, it is evident that $c_1 = 0$, and for vertical flow with fine-textured soils c_1 may perhaps be of negligible magnitude in comparison with c_2 , and equation (a) becomes,

$$a = c_2 (1 - e^{-\beta t}) \quad (b)$$

In figure 6 is shown the graph of water-front, distance, and time data obtained in the laboratory from a rectangular box 6 by 9 cm. in cross-sectional area, one end of which was bent downward and kept permanently in contact with

free water maintained at a constant height about 10 cm. below the center of the box. The broken line represents a typical curve of the family (b), whereas

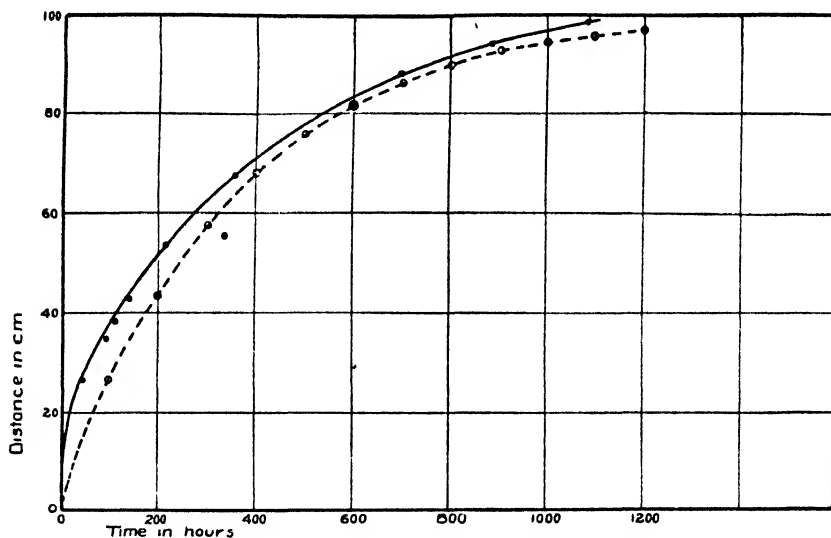


FIG. 6. THE DISTANCE OF THE WATER FRONT FROM THE SOURCE OF SUPPLY AS A FUNCTION OF THE TIME

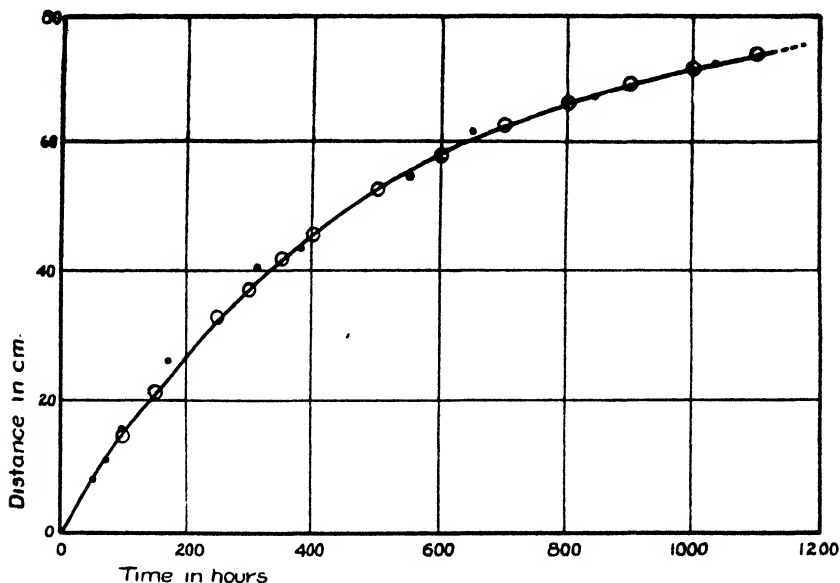


FIG. 7. A PART OF THE DATA OF FIGURE 6 WITH THE ORIGIN OF COÖRDINATES SHIFTED ALONG THE CURVE

the full line with black dots represents the experimental data. It is evident, however, that equation (b) would hold only from the end of the saturated column (fig. 10), and while the experimental data were not conclusive on this

point, it is to be expected that the end of the column nearest the source of supply should become completely saturated for a limited distance. In figure 7 is shown a part of the data of figure 6 with the origin shifted along the curve an arbitrary short distance, and it may be seen that the theoretical points fall almost precisely on the transformed curve.

In figure 8 are plotted curves representing the average values for similar distance-time data for a series of duplicate vertical tubes where the moisture was allowed to move up in the one case and down in the other from soils of varying moisture content into dry soil (B, fig. 10). The data for these curves were obtained from the published work of Harris and Turpin (5). The constants of equation (b) were determined for each of the several curves of the series and the theoretical curves are shown as in the other cases. It is evident here that neglecting the correction term above mentioned gives rise to a considerable difference in theoretical and experimental curves for small values of t . It should also be observed that the asymptote will change slowly with the time ultimately becoming infinite, since the equation was integrated assuming that slowly varying coefficients were constant.

Another case of interest may be noted. If we consider the amount of moisture Q in a given section of soil (C, fig. 10) as a function of the time, we may write for the quantity of moisture in a section of unit area and length a ,

$$\begin{aligned} Q &= \int_0^a \rho dx, \frac{\partial Q}{\partial t} = \int_0^a \frac{\partial \rho}{\partial t} dx = \int_0^a -Kc \left(\frac{\partial^2 \rho}{\partial x^2} - \frac{2\rho}{c} g \frac{\partial \rho}{\partial x} \right) dx \\ &= -K \left\{ c \left[\frac{\partial \rho}{\partial x} \right]_{x=a} - \frac{\partial \rho}{\partial x} \right]_{x=0} - g [\rho_a^2 - \rho_0^2] \} \end{aligned}$$

If we assume that

$$\rho = \rho_0 + \alpha e^{-\beta t}$$

where ρ_0 is independent of x and t and α is independent of t , and make this substitution, we obtain

$$\begin{aligned} \frac{\partial Q}{\partial t} &= -K \{ c e^{-\beta t} (A_a - A_0) - 2 g \rho_0 e^{-\beta t} (\alpha_a - \alpha_0) - g e^{-2\beta t} (\alpha_a^2 - \alpha_0^2) \} \\ &= -K e^{-\beta t} \{ c (A_a - A_0) - 2 g \rho_0 (\alpha_a - \alpha_0) - g (\alpha_a^2 - \alpha_0^2) e^{-\beta t} \} \end{aligned}$$

and

$$Q = \kappa' + L e^{-\beta t} + M e^{-2\beta t} \quad (V)$$

where κ' , L , and M are constants. The asymptote κ' is the lento-capillarity constant as *measured* by one of the authors (10). It is evident, however, that it is in reality a measure of the equilibrium moisture density immediately below the stratum affected by evaporation, which may be far above the amount of the residual "solid" film adhering to the surface of the soil grains. Strictly speaking, the term should designate this residual film. The meaning of L and M will no doubt be evident from the integration.

In figure 9 are plotted the mean values of the water content in the first 6 feet of a field plot at the Greenville Experiment Farm as a function of the

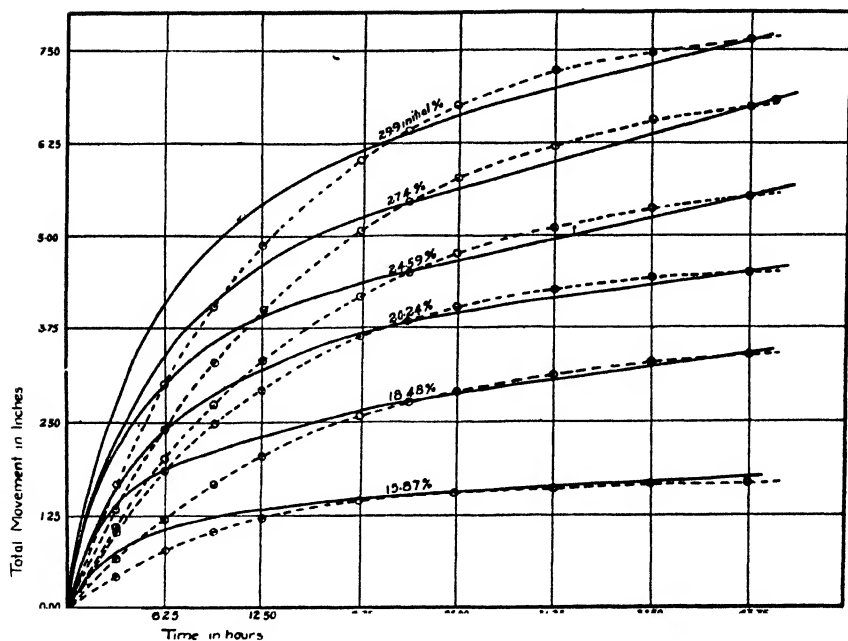


FIG. 8. THE DISTANCE OF WATER FRONT AND TIME DATA WHERE WATER WAS ALLOWED TO MOVE INTO DRY SOIL FROM MOIST SOIL OF VARYING MOISTURE CONTENT

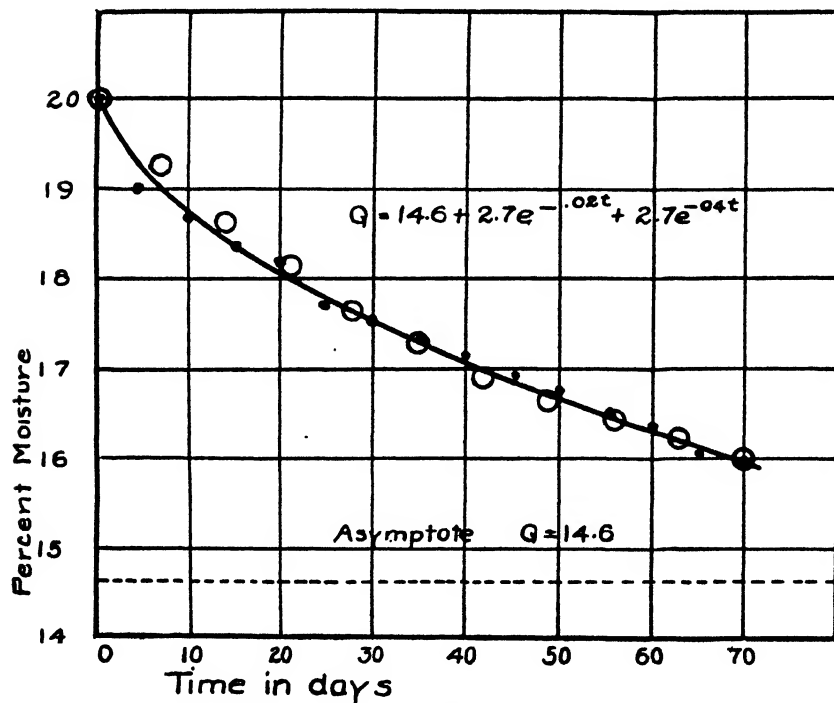


FIG. 9. THE DOWNWARD MOVEMENT OF MOISTURE IN A FIELD SOIL

The ordinate represents the average moisture content in the first 6 feet of soil expressed as per cent on the dry basis, the abscissa represents time in days from the time of maximum moisture content.

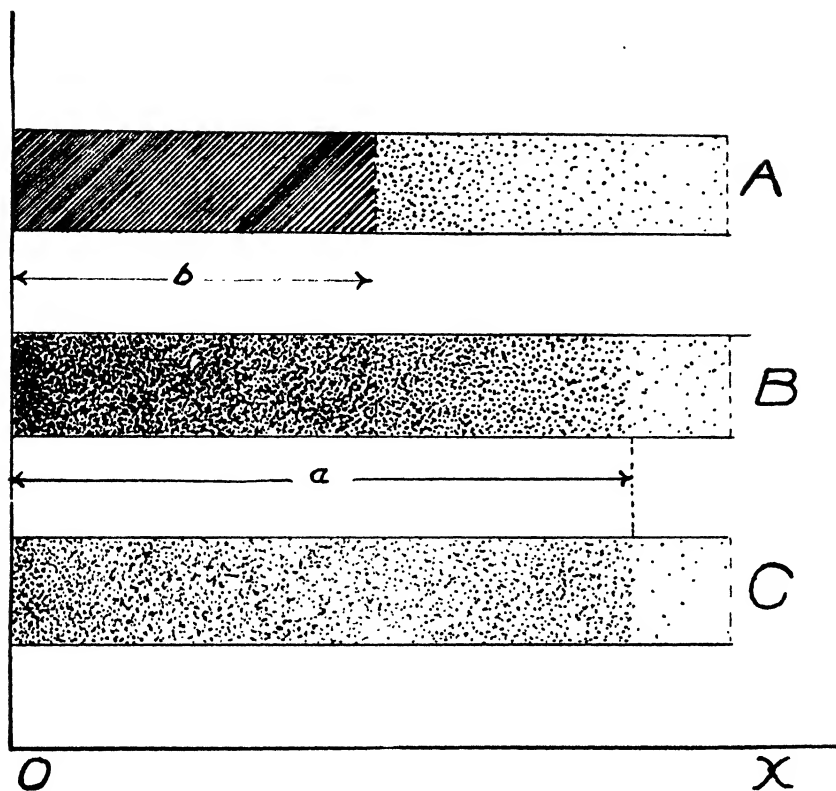


FIG. 10. DIAGRAM OF SOIL COLUMNS OF VARYING MOISTURE CONTENT

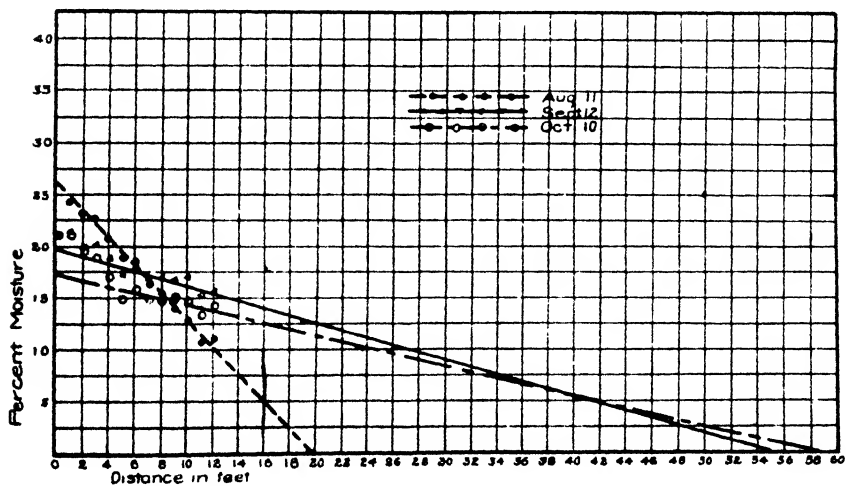


FIG. 11. THE EXTRAPOLATED CURVES APPROXIMATING THE MOISTURE DISTRIBUTION IN A HORIZONTAL BOX WHERE MOISTURE WAS ALLOWED TO MOVE HORIZONTALLY
Three curves are shown corresponding to different dates over a 70-day period

time after a long continued thorough sprinkling of the plots. The theoretical values derived from (V) are indicated as in the previous cases. It is apparent also in this case that regardless of its theoretical significance equation (V) is in harmony with the experimental facts.

The constant β is the product of the diffusion constant k ($= Kc$) and the reciprocal of the distance from the extrapolated saturation point to the extrapolated point of zero moisture content. This distance was not constant in this experiment but ranged from about 20 to 60 feet during the 70-day interval, as may be seen from the slope curves shown in figure 11 for the extremes of this time interval. If we use 40 feet as an approximate average, we obtain, after reducing to c. g. s. units, $k = -2.7 \times 10^{-3}$, which is consistent with values previously determined (3).

III. CONCLUSION

Two assumptions have been made in the development and integration of a general equation for the movement of moisture through an *ideal* soil, viz.,

(a) The inherent moisture conductivity in such a soil is independent of the moisture content.

(b) The capillary potential is a linear function of the reciprocal of the moisture content.

The general equation has been solved for various special cases and experimental results obtained from ordinary soils have been shown supporting the general theory.

It is not held, however, that the results are wholly conclusive. Departures from the general equation and its integrated forms that may arise in future investigations may be traced to one or the other of the two assumptions named. The theoretical material is presented, however, primarily as a working hypothesis, with the hope that those who may be interested will assist in the perfection of the methods of investigation of these and correlated problems.

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SOME STUDIES ON THE RATE OF FORMATION OF SOLUBLE SUBSTANCES IN SEVERAL ORGANIC SOILS

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INTRODUCTION

Researches concerning the rate of formation of soluble salts in a great many mineral soils have been made, but little is known of the behavior of organic soils under similar conditions. It is the purpose of this paper to present the results of a preliminary study of such activities in seven organic soils. The work includes the following considerations:

A. (1) Relative solubility (solubility is taken to mean the power of soil to form soluble substances) at different temperatures. (2) Relative solubility under different moisture conditions.

B. (1) Relative solubility of different depths of the soil section under optimum moisture conditions.

GENERAL PROCEDURE

The freezing-point method for the determination of soluble material in the soil solution, as devised by Bouyoucos (1) was employed as the means of studying the changes in concentration. The samples were always frozen while saturated with water. Freezings were made in duplicate, but in some cases difficulties were encountered in obtaining concordant results. The samples were aerated regularly while in storage. A constant temperature of 25°C. was maintained in a large chamber equipped with heating coils and a thermostat, while a temperature of 7°C. was obtained by means of a ventilated cold room used for the cold storage of fruits.

The soils used in the work discussed under section A of this paper were surface samples, while those described in section B were procured by digging a pit having one perpendicular wall from which blocks of soil could be removed at suitable depths.

DESCRIPTION OF SOILS

1. *Mt. Hope*. A very low lying deposit formed largely from woody accumulations, but having a well decomposed surface of about 8 inches in depth, which was covered with blue-grass sod. Drainage was found to be rather poor, and the lower sections were mostly partially decayed trees and stumps.

At about 40 inches a shallow marl deposit was found. The water-table ordinarily occurs about 12 inches from the surface.

2. *Shaw*. This soil was drained artificially about 15 months previous to sampling and was found to be quite peaty after the upper 5 or 6 inches of friable material had been removed. Weeds and some willows constituted the only vegetation. The deposit seemed to have been formed by the accumulation of mosses and sedges, as many leaf forms were distinguishable in the lower levels. The deposit is a deep one, probably exceeding 10 feet in thickness, and the water-table generally stands at approximately 24 inches from the surface.

3. *Farm Lane*. This is a rather shallow deposit, which has been drained and under cultivation for about 25 years. It has about 12 inches of well oxidized material on top, which is quite sticky, but is rather peaty underneath. It was undoubtedly formed from mosses and sedges. The water-table is usually at a depth of 30 inches. At the time of sampling there was an excellent stand of clover and timothy upon it.

4. *Town Line*. A deposit, reaching only about 3 feet in depth, of material well decomposed nearly to the bottom. The drainage is excellent and a meadow sod exists on the soil. The material resembles the Farm Lane sample as to origin.

5. *Chandler*. This body of soil consists of many thousand acres, for the most part well drained. The deposit was under water, however, until about three years previous to the time the samples were taken. It is covered with a blue-grass sod, many willows and some small poplars. The surface is decomposed to a depth of about 8 inches, gradually changing to a very fibrous peat, in which coarse stems of plants and some tree stumps are noticeable. The water-table at the point of sampling was never encountered at the depth of 36 inches.

6. *Plots*. The drainage being poor, the soil is a very fibrous and coarse peat from the surface downward, and supports a scanty growth of poplar and willow, with occasionally patches of blue-grass sod. In some parts of the soil section, poplar roots and other woody substances are found. Decomposition has scarcely begun and water stands at the surface a large portion of each year.

7. *Interurban*. Taken from the roadside near a small swamp stream. Elms flourish upon it as well as some small bushes. The surface 8 inches is quite granular, changing quickly to a very tough layer nearly 10 inches thick which resembles lignite. Water stands at this point. Below this a peaty substance is found and at a depth of about 3 feet a blue, mottled clay occurs. The intractable layer, when dry, becomes so hard, that it is necessary to grind it before it can be passed through a screen. This soil presents an unusual soil section.

EXPERIMENTAL

A. The effect of temperature and moisture upon the formation of soluble material

About 400 gm. of each air-dry soil were placed in large funnels, and leached with 100-cc. portions of distilled water until 700-cc. had run through each filter. This treatment was sufficient to reduce the concentration of the soil solution almost to zero. As each 100-cc. portion of the leachings was collected the freezing-point lowering was determined. The data in table 1 from two of the samples shows the amount of material in each portion.

Thus, at first there is an appreciable quantity of soluble material washed out, but the amount gradually decreases, until a nearly constant loss occurs. The amount remaining in solution in the soils may then be considered to be almost negligible.

TABLE 1
The freezing-point lowerings and parts per million found in the leachings
0.003°C. Depression = 100 p.p.m. (1).

	SAMPLE 1		SAMPLE 2	
	Freezing-point depression	Parts per million.	Freezing-point depression	Parts per million.
	°C.		°C.	
First 100 cc.	0.008	266	0.012	400
Second.	0.010	333	0.008	266
Third.	0.006	200	0.005	166
Fourth.	0.004	133	0.004	133
Fifth.	0.002	66	0.004	133
Sixth.	0.003	100	0.003	100
Seventh.	0.002	66	0.002	66

After drainage had ceased the soils were placed in pans and thoroughly mixed, and six freezing tubes of each saturated soil prepared. The remainder of the soil was allowed to dry in air to an optimum moisture condition. Three of the freezing tubes were stored at 25°C. while the other three were kept at 7°C. The freezing-point depression of each soil was obtained at this point as the initial concentration. The contents of the tubes were frozen after 13, 25, 38 and 50 days had elapsed.

The data obtained are summarized in tables 2 and 3 and graphically shown for soils 1 and 5 in figures 1 and 2. The data in these tables show that in all the soils studied at both temperatures there is a rather rapid rise in the concentration of the solutions for a certain time, after which a decrease occurs. The time required to reach the maximum depression varies with each soil. Also, for any one soil, different temperatures cause variations in the time. Thus soil 1 at 25°C. reached its greatest depression after about 13 days, while at 7°C. it required 38 days to produce nearly the same concentration. This soil, then, required nearly three times as long to attain the crest when kept

TABLE 2

Freezing-point lowerings of soils kept under saturated moisture conditions and at a temperature of 25°C.

SAMPLE	FREEZING-POINT DEPRESSIONS				
	Original	13 days	25 days	38 days	50 days
	°C.	°C.	°C.	°C.	°C
1	0.002	0.050	0.026	0.025	0.015
	0.001	0.053	0.024	0.022	0.017
2	0.002	0.036	0.022	0.010	0.007
	0.003	0.034	0.020	0.014	0.009
3	0.002	0.028	0.022	0.018	0.022
	0.003	0.028	0.020	0.020	0.018
4	0.002	0.038	0.022	0.034	0.033
	0.004	0.040	0.020	0.030	0.029
5	0.002	0.043	0.037	0.020	0.021
	0.002	0.047	0.036	0.024	0.022
6	0.000	0.008	0.014	0.010	0.012
	0.001	0.010	0.014	0.010	0.014
7	0.003	0.062	0.053	0.054	0.043
	0.002	0.058	0.051	0.052	0.045

TABLE 3

Freezing-point lowerings of soils kept under saturated moisture conditions and at a temperature of 7°C.

SAMPLE	FREEZING-POINT DEPRESSIONS				
	Original	13 days	25 days	38 days	50 days
	°C.	°C.	°C.	°C.	°C.
1	0.002	0.036	0.040	0.050	0.042
	0.001	0.040	0.042	0.052	0.045
2	0.002	0.024	0.025	0.022	0.024
	0.003	0.020	0.028	0.024	0.020
3	0.002	0.018	0.018	0.010	0.014
	0.003	0.018	0.020	0.008	0.014
4	0.002	0.032	0.028	0.028	0.016
	0.004	0.028	0.030	0.026	0.014
5	0.002	0.036	0.050	0.024	0.032
	0.002	0.038	0.050	0.022	0.028
6	0.000	0.007	0.015	0.006	0.007
	0.001	0.010	0.013	0.007	0.006
7	0.003	0.042	0.052	0.027	0.028
	0.002	0.038	0.048	0.028	0.028

at the lower temperature. Again, at 25°C. soil 6 required 25 days to rise, as compared with 13 days for soil 1. Soil 6 is a very fibrous peat, and is rather slow in forming soluble material. The similarity which exists between the other five samples and no. 1 with regard to rate of action is worthy of consideration. While each gave a different depression, they were all at their highest point in 13 days. At the lower temperature, the soils vary much more, requiring from 13 days to 38 days to reach the highest concentration. In general, the lower temperature tends to retard the formation of soluble material.

McCool and Millar (2) found somewhat similar results with mineral soils, and attribute the decrease in salt content to either reabsorption, chemical change which produced less soluble materials or to biological activity. It is very probable that in organic soils, the latter is of great importance, as is also absorption. A study of this phenomenon is now in progress in this laboratory,

TABLE 4

Moisture content of soils at the time of the original freezing-point determination, together with the water necessary to obtain saturated conditions

SAMPLE	MOISTURE DURING STORAGE	WATER ADDED TO EACH BOTTLE FOR FREEZING
	<i>per cent</i>	<i>cc.</i>
1	65.78	12
2	63.61	12
3	61.10	12
4	62.19	12
5	66.45	16
6	73.35	25
7	63.15	17

which seems to show that some ions steadily increase in soil solutions while others rise and then decrease in amount. Since the freezing-point method measures only the resultant solubilities, it is not improbable that as new substances are formed with different solubility products the amount of material affecting the freezing point will change constantly. So it may be shown that chemical change also plays its part in varying the salt content of soils, both in amounts and in composition.

When the samples of each soil for the preceding section had been removed the remainder was dried in air until optimum moisture was obtained. Eight sample bottles, each of 150-cc. capacity, and fitted with a stopper, were employed and 40 gm. of soil placed into each one. Also a sample was frozen at once, to provide the original readings. Four bottles of each muck were kept at 25°C. and four bottles at 7°C. After 10, 20, 40 and 60 days, respectively, one bottle of each soil from each temperature was frozen after enough distilled water had been added to make saturated moisture conditions.

These samples were frequently aired while in storage. The moisture percentages at which the soils were stored, and the amounts of distilled water per bottle necessary to obtain saturation are presented in table 4.

TABLE 5

Freezing-point lowerings of soils kept under optimum moisture conditions and at the temperature of 25°C.

SAMPLE	FREEZING-POINT DEPRESSIONS				
	Original	10 days	20 days	40 days	60 days
	°C.	°C.	°C.	°C.	°C.
1	0.003	0.024	0.050	0.033	0.035
	0.001	0.021	0.048	0.032	0.032
2	0.002	0.017	0.044	0.050	0.052
	0.004	0.018	0.042	0.046	0.048
3	0.002	0.021	0.028	0.040	0.020
	0.003	0.019	0.028	0.041	0.024
4	0.007	0.032	0.033	0.062	0.052
	0.009	0.028	0.036	0.059	0.052
5	0.006	0.024	0.060	0.072	0.060
	0.005	0.026	0.056	0.068	0.062
6	0.004	0.008	0.014	0.010	0.006
	0.005	0.010	0.012	0.012	0.006
7	0.005	0.034	0.033	0.038	0.042
	0.007	0.037	0.035	0.036	0.040

TABLE 6

Freezing-point lowerings of soils kept under optimum moisture conditions and at the temperature of 7°C.

	FREEZING-POINT DEPRESSIONS				
	Original	10 days	20 days	40 days	60 days
	°C.	°C.	°C.	°C.	°C.
1	0.003	0.020	0.014	0.018	0.022
	0.001	0.018	0.018	0.018	0.018
2	0.002	0.014	0.016	0.016	0.020
	0.004	0.015	0.015	0.017	0.020
3	0.002	0.010	0.015	0.012	0.008
	0.003	0.012	0.016	0.015	0.011
4	0.007	0.024	0.027	0.027	0.022
	0.009	0.020	0.026	0.026	0.024
5	0.006	0.015	0.014	0.012	0.006
	0.005	0.014	0.014	0.013	0.008
6	0.004	0.006	0.006	0.012	0.004
	0.005	0.005	0.006	0.010	0.006
7	0.005	0.012	0.014	0.014	0.019
	0.007	0.013	0.013	0.017	0.016

The results of the experiment are shown in tables 5 and 6, and the data for soils 1 and 5 are represented by figures 1 and 2.

It is evident that the accumulation of soluble substances is a relatively slow process under lower moisture conditions. The soils have taken much

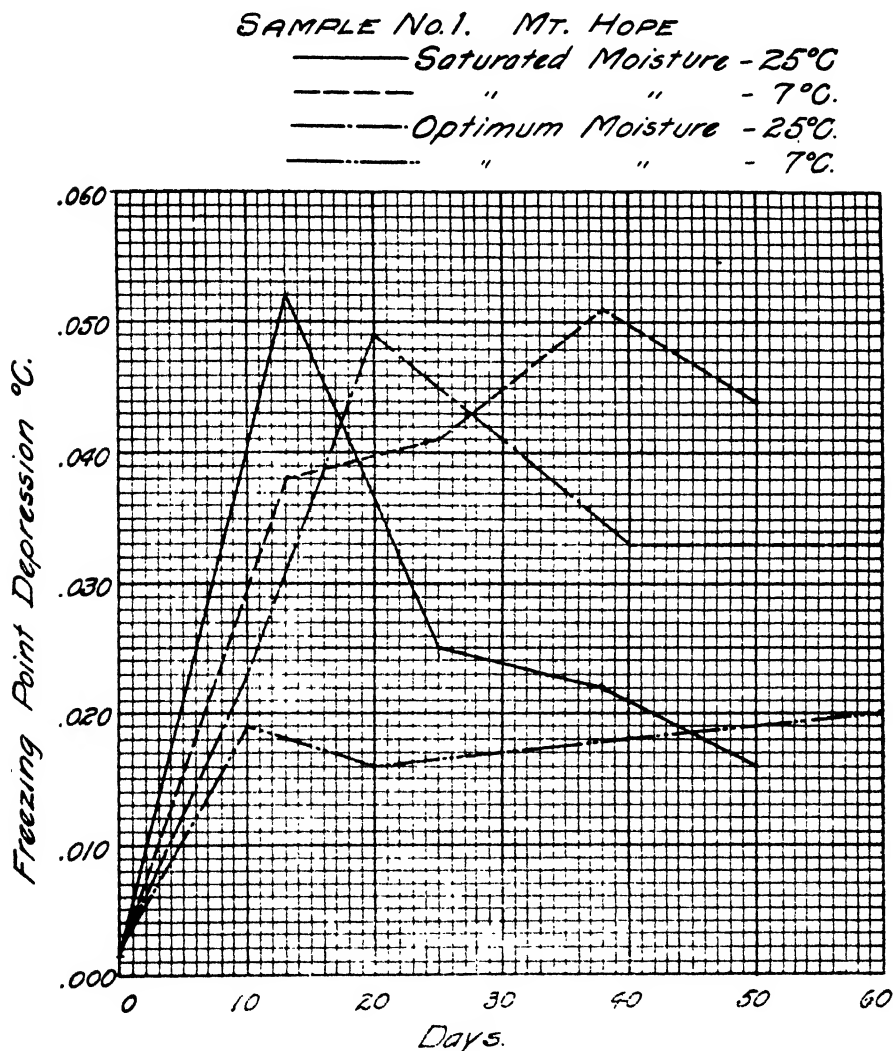


FIG. 1. GRAPH SHOWING THE CHANGES IN CONCENTRATION OF THE SOLUTIONS IN SOIL 1, UPON STANDING UNDER TWO DIFFERENT CONDITIONS OF MOISTURE AND OF TEMPERATURE, FROM THE DATA GIVEN IN TABLES 2, 3, 5 AND 6

longer to reach their highest concentration at optimum moisture than they needed under saturation. In table 5 soils 1 and 6 were highest in 20 days, soils 3, 4 and 5 in 40 days, while no. 2 and 7 were most intense at 60 days. Referring back to the data in table 2, also obtained at 25°C., most of the

samples showed the greatest depression in 13 days. Also, with the exception of soils 1 and 6, larger freezing-point depressions were found in the optimum moisture series, showing that the latter water content produces greater amounts of soluble materials.

SAMPLE No. 5. CHANDLER

———— Saturated Moisture -25°C.
 ----- " " - 7°C.
 - - - - - Optimum Moisture -25°C.
 - · - · - " " - 7°C.

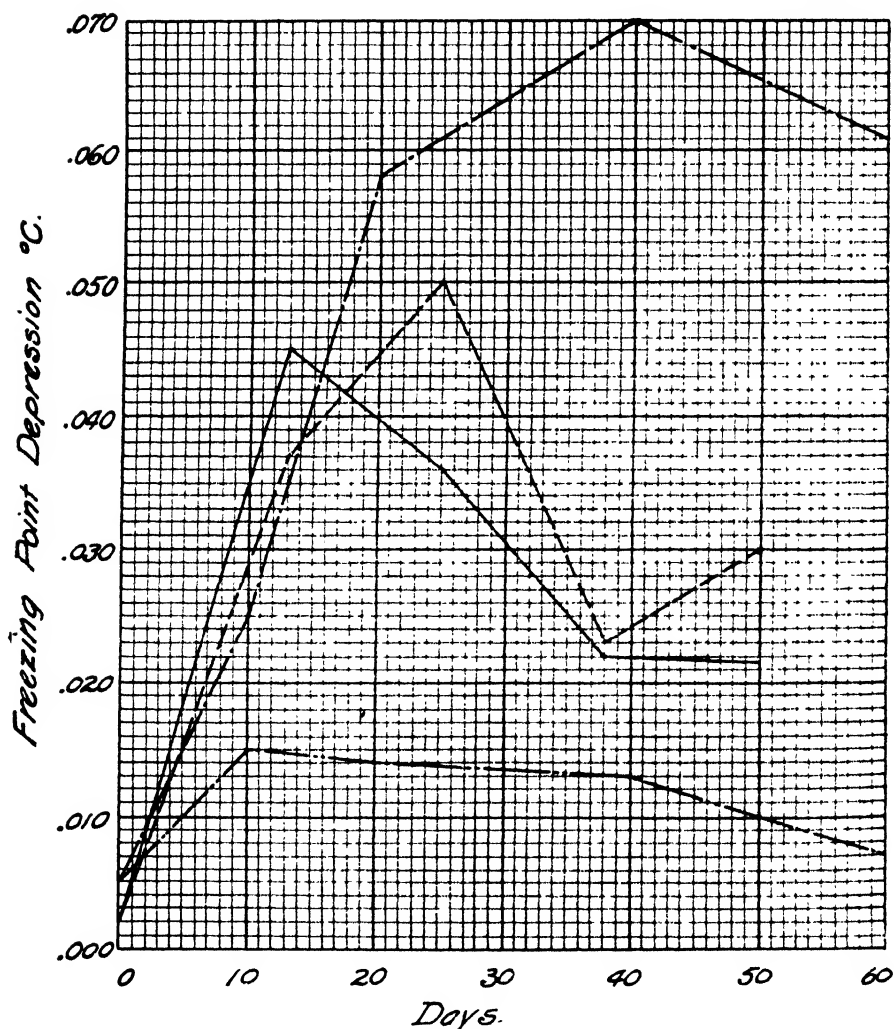


FIG. 2. GRAPH SHOWING THE CHANGES IN CONCENTRATION OF THE SOLUTIONS IN SOIL 5, UPON STANDING UNDER TWO DIFFERENT CONDITIONS OF MOISTURE AND OF TEMPERATURE, FROM THE DATA GIVEN IN TABLES 2, 3, 5 AND 6

As was the case in tables 2 and 3, the lower temperature retards salt formation both in amount and in rapidity. Thus in several soils the maximum readings occur after having stood for 60 days. A very interesting point lies in the comparison of those soils kept at saturated water, and those stored at optimum at 7°C. In all cases, the saturated soils reached a greater concentration than those at the optimum water content. This is in direct contrast to the conditions found in the two series at 25°C. These relations point strongly toward the biological factors, which would find most congenial conditions at a higher temperature with optimum moisture. The facts brought out by the preceding discussions may be stated in general as follows:

At 25°C. the soils produced more soluble material under optimum moisture conditions. Exactly the reverse is true at a temperature of 7°C., at which the saturated soils reached a higher concentration. The higher temperature seems to hasten the rate of formation for all moisture conditions, and saturated moisture increases the rate at both temperatures.

B. Studies of the relative amounts of soluble material formed by the soil sections of different depths

The same soils that were used in the preceding work, were sampled at the following depths, care being exercised to eliminate mixing of the various layers. The first sample included the soil from the surface to a depth of 6 inches, the second ranged from 6 to 12 inches, the third from 12 to 24 inches, and the fourth and last from 24 to 36 inches. The depth of 36 inches was always sufficient to reach well into the material which was but slightly, if any, decayed. Referring back to the description of the soils, it will be seen that the lower section in all cases was below the water-table. The samples were permitted to lose water at ordinary room temperature until they attained the optimum moisture content, when they were screened and thoroughly mixed; then 40-gm. portions placed in bottles and stored in a large chamber, the temperature of which remained at 25°C. Determinations of the soluble material present were made at once, and after having stood 10, 30 and 60 days, respectively. As before, care in aerating the bottles was observed during the storage period. The results obtained are set forth in table 7. These freezings were made by adding the amounts of distilled water given in table 4, which brought each sample to a condition of saturation. As in the first experiments the results for two soils, no. 1 and 5, are shown by means of graphs in figures 3 and 4.

The consideration of the data of table 7 resolves itself into two phases: (a) the comparison between different layers of the same soil, and (b) the comparison of the same layer in different soils.

Regarding the first phase, quite uniform tendencies are shown by the data. Thus in all the soils the surface layer gives rise to a greater concentration than any of the lower layers. In all the samples except no. 1 and 7, the second

TABLE 7

Freezing-point lowerings of soils kept under optimum moisture conditions and at 25°C.

SAMPLE	DEPTH FROM THE SURFACE	FREEZING-POINT DEPRESSIONS			
		Original	10 days	30 days	60 days
	<i>inches</i>	°C.	°C.	°C.	°C
1	0-6	(1) 0.012	0.018	0.023	0.032
		(2) 0.010	0.015	0.019	0.034
	6-12	(1) 0.002	0.012	0.015	0.012
		(2) 0.002	0.010	0.015	0.014
	12-24	(1) 0.008	0.018	0.015	0.007
		(2) 0.006	0.016	0.010	0.008
	24-36	(1) 0.010	0.018	0.015	0.012
		(2) 0.008	0.016	0.015	0.010
2	0-6	(1) 0.010	0.017	0.025	0.028
		(2) 0.010	0.014	0.020	0.026
	6-12	(1) 0.012	0.014	0.027	0.026
		(2) 0.010	0.014	0.032	0.022
	12-24	(1) 0.010	0.010	0.020	0.018
		(2) 0.010	0.009	0.017	0.016
	24-36	(1) 0.008	0.009	0.015	0.013
		(2) 0.006	0.006	0.013	0.010
3	0-6	(1) 0.018	0.024	0.025	0.028
		(2) 0.014	0.028	0.029	0.028
	6-12	(1) 0.006	0.002	0.010	0.012
		(2) 0.003	0.005	0.014	0.010
	12-24	(1) 0.006	0.006	0.006	0.011
		(2) 0.004	0.004	0.010	0.008
	24-36	(1) 0.008	0.006	0.008	0.006
		(2) 0.004	0.006	0.010	0.006
4	0-6	(1) 0.010	0.018	0.023	0.024
		(2) 0.012	0.020	0.026	0.025
	6-12	(1) 0.008	0.018	0.020	0.019
		(2) 0.008	0.016	0.021	0.020
	12-24	(1) 0.004	0.009	0.012	0.012
		(2) 0.004	0.012	0.012	0.012
	24-36	(1) 0.004	0.004	0.008	0.006
		(2) 0.003	0.006	0.006	0.005

TABLE 7—Continued

SAMPLE	DEPTH FROM THE SURFACE	FREEZING-POINT DEPRESSIONS			
		Original	10 days	30 days	60 days
	<i>inches</i>	°C.	°C	°C.	°C
5	0-6	(1) 0.018	0.022	0.045	0.042
		(2) 0.016	0.024	0.049	0.038
	6-12	(1) 0.010	0.018	0.040	0.027
		(2) 0.009	0.014	0.040	0.024
	12-24	(1) 0.006	0.010	0.031	0.012
		(2) 0.005	0.009	0.028	0.010
	24-36	(1) 0.006	0.006	0.013	0.007
		(2) 0.004	0.006	0.015	0.004
6	0-6	(1) 0.012	0.018	0.028	0.024
		(2) 0.008	0.014	0.032	0.026
	6-12	(1) 0.008	0.008	0.013	0.006
		(2) 0.006	0.006	0.016	0.004
	12-24	(1) 0.004	0.002	0.008	0.004
		(2) 0.002	0.001	0.008	0.003
	24-36	(1) 0.004	0.002	0.006	0.002
		(2) 0.003	0.005	0.010	0.003
7	0-6	(1) 0.013	0.028	0.048	0.052
		(2) 0.011	0.032	0.052	0.052
	6-12	(1) 0.002	0.028	0.040	0.040
		(2) 0.005	0.026	0.037	0.042
	12-24	(1) 0.010	0.020	0.045	0.037
		(2) 0.006	0.018	0.042	0.034
	24-36	(1) 0.006	0.018	0.020	0.022
		(2) 0.004	0.016	0.020	0.024

layer produces the next highest depression, with the soils below 1 foot in depth, showing but little activity in forming soluble material.

Soil 1 gave consistently higher results in the lowest section over those directly above, the surface being the only portion to outstrip it. This is probably due to the presence of marl just below 3 feet in depth. Sample 7 shows great activity in its third division from the top, which can be explained only by the variation in deposits.

Most deep mucks show a general diminution in the production of soluble substances, under optimum moisture conditions, with increasing depths. This has been found to be true in mineral soils to a large extent. It appears that soil materials which are below the active zone of weathering are more

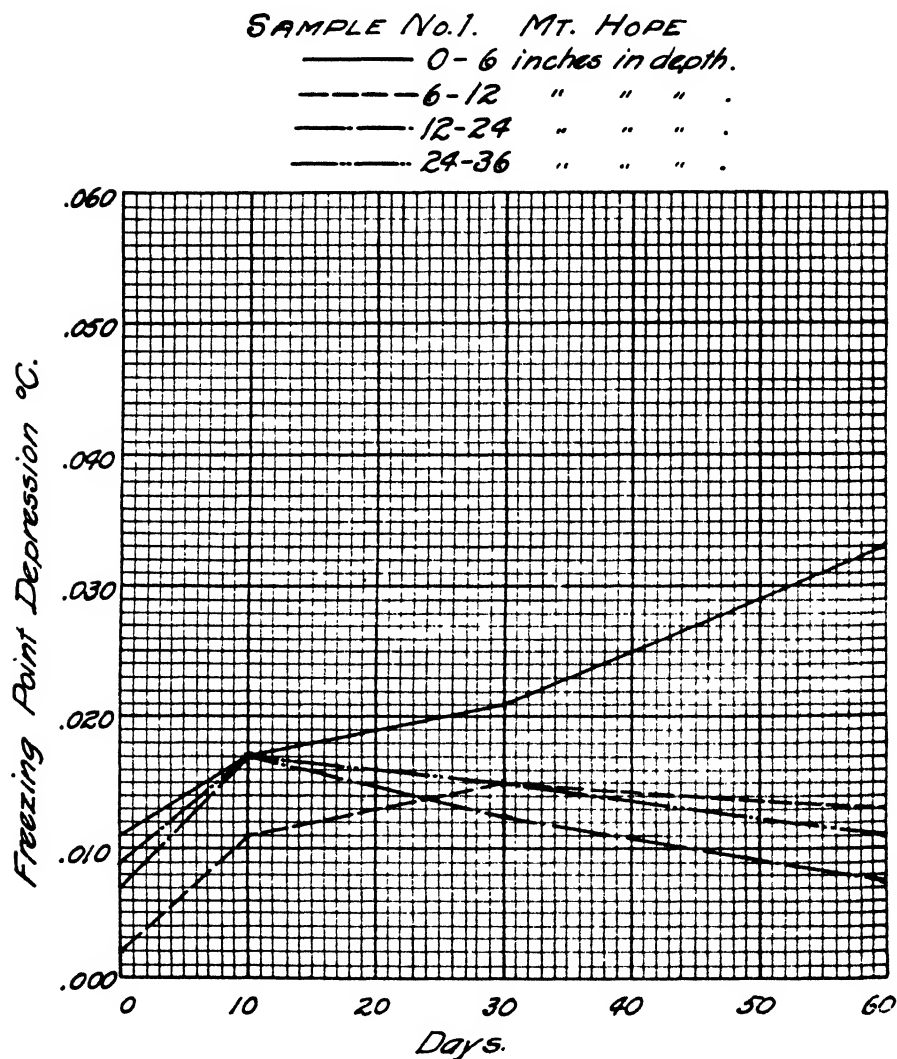


FIG. 3. GRAPH SHOWING THE CHANGES IN CONCENTRATION OF THE SOLUTIONS IN DIFFERENT LAYERS OF SOIL 1, UPON STANDING UNDER OPTIMUM MOISTURE CONDITIONS AND AT A TEMPERATURE OF 25°C.

inert, and would support plant growth with difficulty, if at all. Thus, burning of the surface layers of mucks would probably destroy the most active portions of the soil, and leave the almost inert, unweathered material as the medium for plant growth. Theoretically this might result in crop failure for a few

years until weathering had again made an active layer or one which formed soluble constituents readily. The reasons for the higher solubility of surface layers must lie largely in the realms of biological activity and in chemical decomposition.

SAMPLE No. 5. CHANDLER

— 0-6 inches in depth.
 - - - 6-12 " " "
 — · — 12-24 " " "
 - · - · - 24-36 " " "

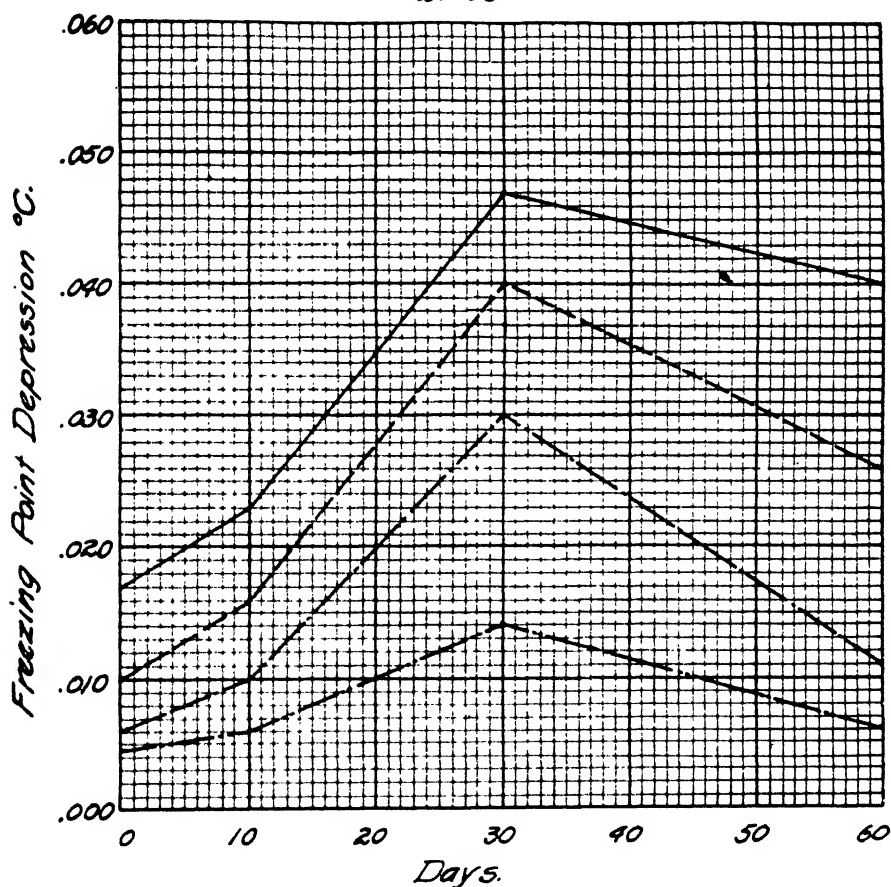


FIG. 4. GRAPH SHOWING THE CHANGES IN CONCENTRATION OF THE SOLUTIONS IN DIFFERENT LAYERS OF SOIL 5, UPON STANDING UNDER OPTIMUM MOISTURE CONDITIONS AND AT A TEMPERATURE OF 25°C.

When a comparison of the same layers in different soils is undertaken, it is found that many of the facts already shown in table 5 are repeated with regard to the surface soils. The order of magnitude of the freezing-point depression for the surface soils is: no. 7, 5, 1, 3, 2, 6 and 4, with the last four

closely grouped. Table 5 shows the highest depressions in soils 7, 5 and 1. as above.

The second layers give results quite similar to those for the surface, being always of somewhat lower magnitude, however.

There are striking similarities in all these soils, in the third and fourth sections, variations of only a few thousandths of a degree Centigrade occurring throughout. For the deposits studied almost regardless of their origin, the material below one foot in depth, shows marked similarities in the amount of soluble constituents. The variation in the relative fertility of muck soils must be caused by the subsequent weathering of this parent mass, which changes some organic remains into more soluble compounds than other debris produces.

An interesting comparison may be made with regard to the depth of weathering of organic and mineral soils. This work shows that in these mucks there has been little climatic action below a depth of 2 feet, while even our most recent mineral soils of fairly heavy texture have weathered from 3 to 6 feet deep, and sandy soils are frequently acted upon even deeper.

There would be a greater zone where the soil materials are more soluble, in mineral soils, and this may have some bearing upon the excessive fertilizer requirements for mineral elements in muck soils.

SUMMARY

1. By means of the freezing-point method (used by Bouyoucos and others), the study of the relative activities of seven organic soils under different moisture and temperature conditions, and at different depths was carried out.

2. At any given moisture content, the effect of a higher temperature is to increase the rate of formation of soluble material, and conversely, lower temperatures decrease the rate of formation.

3. For the higher temperatures, optimum moisture conditions tend to bring greater amounts of material into solution than are found under saturated water conditions.

4. Exactly the opposite effect was observed with lower temperatures.

5. Generally moist soils upon standing increase in concentration to a certain point, after which a decline occurs. This is probably due to the following causes: (a) reabsorption, (b) chemical change to less soluble compounds, or (c) biological activity.

6. Organic soils vary at different depths in the amount of soluble substances present.

7. Different depths also vary in the rate and amount of material made soluble upon standing.

8. Below a depth of 2 feet, the muck soils studied are very inactive.

9. The surface layers usually produce the bulk of the soluble plant-foods.

10. In general the ability to yield soluble materials decreases regularly from the surface to the water-table.

11. The zone of weathering and the region of greatest activity closely coincide.

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THE NON-BIOLOGICAL OXIDATION OF ELEMENTAL SULFUR IN QUARTZ MEDIA

A PRELIMINARY REPORT

W. H. MACINTIRE, F. J. GRAY AND W. M. SHAW

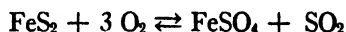
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The conversion of native organic sulfur into sulfates in soils is generally considered to be almost exclusively a biological process. The oxidation of added elemental sulfur is likewise usually attributed to the action of bacteria. The native organic sulfur phase of sulfate generation, as influenced by calcic and magnesian materials in varying amounts, has been under investigation at the University of Tennessee Agricultural Experiment Station since July, 1914. At that time, 46 lysimeters were installed and filled with Cumberland loam, 23 tanks having soil alone, and 23 having surface soil above a 1-foot layer of clay subsoil. Each annual aggregate of sulfate leachings has been determined quantitatively. Divergent effects of calcic and magnesian compounds upon the sulfate outgo, during the first two years, were noted and reported upon in a preliminary paper by the writer and associates (2) in 1917. The supplemental problem of sulfur additions to a Cherokee sandy loam was begun in August, 1917. Fifteen tanks received sulfur additions. Each five-tank group received one of the three forms of sulfur, namely; iron sulfate, iron pyrite, and flowers of sulfur, each in an amount equivalent to a rate of 1000 pounds of sulfur per 2,000,000 pounds of soil. The question of the influence of lime and magnesia upon added sulfur was also included in the supplementary study. In this second installation, comprising a total of 22 lysimeters, the loss of sulfur, as leached sulfates, was determined for each tank periodically, as necessitated by the unsupplemented rainfall. The data secured from the leachings demonstrated that the flowers of sulfur and iron pyrite were both converted into sulfates, with distinct rapidity.

It was at first assumed that the oxidation of both the elemental sulfur and that of pyrite was induced in the main, if not solely, by organisms. However, some doubt was introduced concerning this assumption about two years after the inauguration of the experiment. At that time it was observed that a strong odor of SO_2 was given off from the reserve sample of iron pyrite. This reserve sample had been kept in the dark in an 8-ounce glass bottle, tightly stoppered with an ordinary no. 6 cork stopper. Examination of the pyrite showed that it contained a large amount of SO_4 . A 10-gm. charge was found to yield soluble sulfate of iron, equivalent to a determined weight of 0.4172 gm.

of BaSO_4 , as an average of seven determinations. A search of the literature revealed that the same observation had been made by Allen and Johnston (1) reported in 1910. These workers further reported that an increase of 100 per cent of sulfate of iron was caused by dry grinding for a period of 1 hour. They accounted for the reaction through the following equation:



Contact of moist pulverized metallic iron and flowers of sulfur was found to produce iron sulfide, which reaction was also found to be of record (3). These observations suggested the possibility that the applied elemental sulfur might combine to an extent, with the iron of the soil, forming iron-sulfur compounds which, in turn, would undergo oxidation to sulfates. It even seemed plausible to assume that the presence of iron in some form might be essential to the extensive conversion of elemental sulfur into sulfates.

These findings and observations led to a laboratory study of the two major queries:

1. What function, if any, does metallic iron, and what function does iron oxide, or oxides, have upon the conversion of elemental sulfur to sulfates in soils?
2. Will the effects possibly induced by iron, or its oxides, be independent of biological activation?

It was, therefore, planned to study the oxidation of elemental sulfur in the absence of appreciable quantities of iron, under aerobic and anaerobic conditions, with the unaltered medium, the sterilized medium, and the medium plus inoculation. It was decided to use the purest obtainable quartz as the medium for sulfur additions. An unsuccessful attempt was made to secure an iron-free quartz. The finely ground New England quartzite utilized ran 99.28 per cent SiO_2 , 0.34 per cent Fe_2O_3 and 0.0096 per cent S. The purest hydrogen-precipitated Fe obtainable was used as one source of iron. This material carried 0.0475 per cent of sulfur. The other iron compound used was limonite, analyzing 39.50 per cent iron and 0.013 per cent soluble sulfate sulfur. A futile attempt was made to obtain a sufficient quantity of siderite as another source of iron.

Five-hundred cubic centimeter Pyrex flasks were used as containers for the treated media. The unleached quartz was used in the constant amount of 250 gm., with 14 per cent distilled water additions for moisture. Each treated medium was kept in the dark for a period of 60 days after treatment. The quartz was very finely ground. In addition to the constant amount of 250 gm. of quartz, the following respective single or combined constants were used: 0.1251 gm. of sulfur; 10.0806 gm. of metallic iron; 25.3164 gm. of limonite; 0.5076 gm. of "c.p." precipitated calcium carbonate; 0.5000 gm. of "c.p." precipitated magnesium carbonate; 0.5181 gm. of 100-mesh limestone; and 0.5449 gm. of 100-mesh dolomite. The calcic and magnesian materials were chemically equivalent and were introduced in order to prevent the accumu-

lation of any generated acids, rather than upon the assumption of any direct influences.

The biological conditions maintained in the original quartz-medium experiment were: (a) unaltered quartz, (b) quartz sterilized by heat, (c) inoculation by soil infusion "A," (d) inoculation by soil infusion "B." These four conditions were maintained under both aerobic and anaerobic conditions. The aerobic flasks, both sterile and non-sterile, were stoppered with cotton plugs. The anaerobic atmosphere was produced by a 6-hour passage of purified CO_2 . An additional series containing purified hydrogen also was subjected to experimental treatment, but this series is not yet ready for detailed report. However, it may be stated that the formation of sulfates, in considerable amounts, has occurred in the hydrogen atmosphere.

The sterilization was effected by three successive daily heatings in the autoclave, without contact of quartz and the separately sterilized materials used in the several treatments. The added materials were mixed throughout the dry quartz immediately before the addition of the constant moisture content. All of the flasks were put away in the dark, in a room relatively free of fumes, for a period of 60 days. At the end of the 60-day period the contents of the flasks were extracted by addition of cold distilled water to near-complete flask volume. After 4 hours' shaking and over-night standing, the extracts were filtered through Büchner funnels with double filters. Each residue was then thoroughly mixed and returned to its original flask for an additional period of contact of 40 days, after which the filtration was repeated. The filtrates were analyzed for sulfides, and if deemed necessary, NaOH was introduced. They were then acidified and evaporated to dryness, in order to remove SiO_2 . The engendered sulfates, as well as the sulfates of all blanks, were determined gravimetrically. Tests were made to insure the fact that the precipitations were not BaF_2 . In addition to the eight series of 15 flasks each, one additional set was run simultaneously. This supplemental set contained 12 flasks. Three flasks contained inoculated quartz and nitrate nitrogen to the extent of 10 mgm. of nitrogen, one flask containing sodium nitrate, one calcium nitrate, and one magnesium nitrate. These three nitrate treatments were duplicated with an increase of nitrate nitrogen to a basis of 50 mgm. The six flasks, above described, were then duplicated as to nitrogen treatment, but with the addition of 10.0806 gm. of metallic iron to each flask.

The different treatments given in each of the 15-unit sets, with both aerobic and CO_2 atmospheres and under the four conditions of moist contact, original quartz, sterilized quartz, quartz with inoculation "A" and quartz with inoculation "B," are listed below.

Sulfur alone

Sulfur plus CaCO_3

Sulfur plus limestone

Sulfur plus MgCO_3

Sulfur plus dolomite

Sulfur plus metallic iron
Sulfur plus iron plus CaCO_3
Sulfur plus iron plus limestone
Sulfur plus iron plus MgCO_3
Sulfur plus iron plus dolomite

Sulfur plus limonite
Sulfur plus limonite plus CaCO_3
Sulfur plus limonite plus limestone
Sulfur plus limonite plus MgCO_3
Sulfur plus limonite plus dolomite

It is not intended, at this time, to enter into a detailed discussion of the results. However, a few of the salient findings will be mentioned. In each one of the sixty aerobic treatments there was evidenced a substantial increase in leachable sulfates. The four quartz-sulfur-only checks gave an average increase of 222.5 pounds of sulfate sulfur per 2,000,000 pounds of solid medium, for the four conditions with access to the atmosphere. The four calcic and magnesian materials supplementing sulfur additions in the first group, gave an increase of 396.3 pounds of leached sulfur, as an average of sixteen determinations for the four conditions of the medium. The depressive tendency of metallic iron was evidenced in all of the twenty flasks of the second aerobic group. The average increase in leached sulfate sulfur from these five treatments, under the four experimental conditions, which involved twenty flasks, amounted to 109.5 pounds per 2,000,000 pounds of medium. In the third aerobic group, wherein all twenty flasks contained limonite, an average increase of 475.2 pounds of leached sulfate sulfur was obtained, as calculated to a basis of 2,000,000 pounds of medium. When averaged, the fifteen flasks, to all of which sulfur was added either alone or supplemented by the specified supplementary materials, gave increases of sulfate sulfur amounting to 418.9 pounds, 184.5 pounds, 331.1 pounds, and 329.2 pounds, respectively, for the unaltered quartz, the heat-sterilized quartz and the quartz which received two separate inoculations, "A" and "B."

In the CO_2 -atmosphere series, the four flasks containing only sulfur and quartz gave an average increase of 147.3 pounds of sulfate sulfur from the four experimental conditions of contact. The sixteen quartz-sulfur-carbonate flasks yielded an average increase of soluble sulfate sulfur amounting to 208.9 pounds per 2,000,000 pounds of quartz. A very striking set of results was obtained from the metallic iron group under the anaerobic conditions. In each one of the twenty flasks, a minus quantity of sulfates was recovered. That is, the amount recovered in each instance was actually less than in the corresponding checks of quartz alone and quartz plus the single or combination treatments, including sulfur. When averaged, the twenty consistent minus quantities, ranging between -8.2 pounds and -50.8 pounds, gave -30.3 pounds as representing the depressive action of the metallic iron. The third, or limonite, group in the sealed CO_2 atmosphere gave, as an average of

five flasks for each of the experimental conditions, 305.4 pounds, 319.8 pounds, 126.0 pounds and 139.0 pounds for the unaltered quartz, sterilized quartz and quartz with inoculations "A" and "B," respectively. As an average of the entire fifteen flasks receiving the different additions, under each of the four experimental conditions, the increased outgo of sulfate sulfur amounted to 184.5 pounds, 134.5 pounds, 83.4 pounds, and 95.3 pounds, respectively, for the unaltered quartz, the sterilized quartz and the quartz with inoculations "A" and "B." Corresponding increases of 295.6 pounds, 241.7 pounds, 139.4 pounds and 157.7 pounds were obtained by the omission of the several constant minus results obtained from the metallic iron group; that is, averaging only the increases, which were obtained in every case in the absence of metallic iron.

In the case of the supplementary inoculated CO_2 -atmosphere nitrate series, the smaller nitrate additions failed to show any extensive increase in leached sulfates, above that given by the quartz-sulfur checks, after a 60-day period of contact. On the other hand, the larger nitrate additions exhibited a distinct and consistent depressive influence for each of the three added nitrates. Again in the six flasks containing nitrates and metallic iron, the same characteristic depressive tendency of iron was demonstrated. After the leaching of the nitrates, along with the soluble engendered sulfates, a further contact period of 40 days gave a decided gain in sulfates where both quantities of nitrate nitrogen were added alone; but the minus-quantity characteristic of the metallic iron treatment still obtained in those flasks where both nitrates and iron were introduced.

These results appear positively to establish certain facts. Elemental sulfur will oxidize upon moist contact with relatively pure quartz under both aerobic and anaerobic conditions, as represented, respectively, by access to air through cotton plugs and contact with a sealed atmosphere of purified CO_2 . A distinct reducing and inhibitive effect is manifested by metallic iron under aerobic conditions, with a still greater similar tendency in the atmosphere of CO_2 . Limonite, on the contrary, exhibits a strong tendency to accelerate oxidation when used alone and when supplemented by the several carbonates. Furthermore, the single additions of the several carbonates to the quartz-sulfur mixture resulted in a marked increase in the amounts of sulfates recovered under the aerobic conditions.

In the case of the supplementary nitrate series, the presence of the several nitrates failed to alter the depressive tendencies of metallic iron. In fact, the increase in salt-concentration exhibited an independent depressive action in the absence of the metallic iron. This observation relative to the influence of the concentration of salts in the moisture of the several media, parallels that of the depressive tendency of precipitated MgCO_3 , when its solubility in water was materially increased through contact with an excess of CO_2 .

The chemical explanations of the several observed phenomena will be considered in the more detailed papers to be offered shortly. However, it might

be pointed out at this time that oxygen is readily furnished to the added elemental sulfur through the agency of limonite and through contact with each of the four separate carbonate treatments, where access to air is permitted through cotton plugs. Were the oxidation assumed to be caused as a result of aeration, the added treatments would still necessarily be considered as accelerators. However, the same tendencies have been found to manifest themselves in atmospheres of purified CO_2 and purified hydrogen, which would militate against the aerobic assumption. Again, in the anaerobic flasks, oxygen is available only from water, SiO_2 , limonite, carbonates and the CO_2 gas; unless it be assumed that there occurs upon the surface of the quartz a condensed film of oxygen, or air, not replaced by CO_2 gas, nor by hydrogen; but, nevertheless, available for oxidation of the elemental sulfur under conditions of intimate moist contact. An effort is now being made to obtain some light upon this point. Studies are being made also upon hypothetical by-products such as carbon monoxide, hydrogen, ferrous oxide and others.

It should be stressed that our experiments were not designed to demonstrate that native organic soil sulfur, or added elemental sulfur, is not converted into sulfates through biological activities. Rather, an attempt was made, to determine, as it appears to be definitely established, whether the oxidation of elemental sulfur into sulfates may be induced solely by chemical reactions within a siliceous medium, or media; and, furthermore, what part iron and iron oxide may have in inducing the reactions both alone and with supplementary alkali-earth carbonates.

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THE AMOUNT OF UNFREE WATER IN SOILS AT DIFFERENT MOISTURE CONTENTS

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INTRODUCTION

It has been shown that the results yielded by the freezing-point (2) and dilatometer (1) methods reveal a most intimate and complex relationship between soil and water and necessitate a new classification of the soil moisture on a more logical, real and scientific basis. The new classification that has been revealed by the results of the above methods is as follows:

Gravitational water

Free water

Unfree water $\left\{ \begin{array}{l} \text{capillary-adsorbed} \\ \text{combined} \left\{ \begin{array}{l} \text{water of solid solution or} \\ \text{water of hydration} \end{array} \right. \end{array} \right.$

The free water is that which freezes readily at slightly below zero; while the unfree water is that which freezes finally at the supercooling of -4°C . and also in the temperature of -78° minus the free water. The capillary-adsorbed water, which is part of the initial total unfree water, may become free by certain treatments of the soil, notably repeated freezing and thawing, while the combined water, which is also part of the total unfree water, seems not to be affected by such treatments.

When the freezing-point lowering of soils is determined at different moisture contents it is found that the freezing-point depression increases at an abnormally greater rate than the moisture content decreases. Indeed, the results show that the depression tends to increase in a geometric progression as the moisture content decreases in an arithmetic progression. These facts would seem to indicate that the amount of unfree water increases as the total moisture content decreases. In offering originally an hypothesis to explain the results, it was postulated, however, that the amount of water which the soils cause to become unfree is rather definite at any one moisture content, although part of this unfree water can be rendered free by successive freezing and thawing. This postulate seemed later to be verified by the dilatometer method results which showed that the amount of water which failed to freeze in any one soil was practically the same irrespective of the moisture content.

Keen (3) of the Rothamsted Experiment Station, in a very thorough and intelligent critique of the results yielded by the freezing-point and dilatometer methods together with those that he obtained himself on the evaporation of water in soils, has pointed out that according to mathematical treatment of the experimental data the amount of water rendered unfree by the soil appears not to be constant but varies with the total moisture content. He has applied mathematics to the experimental data and has arrived at the conclusion that the proportion of free to total water continually decreases and that of unfree to total continually increases as the total moisture diminishes in amount. This conclusion is contrary to the original postulate proposed, which appeared to be confirmed by experimental data.

Keen is to be highly commended in his efforts to treat experimental results on a quantitative basis, for the more we can express experimental data in soils mathematically the more we may be able to reduce the subject of soils to an absolute science. Unfortunately, however, soil is such a heterogeneous and complex mass with so many unknown and unforeseen factors that it is not always safe to arrive at or predict conclusions mathematically.

Since a correct knowledge on the subject of the quantity of unfree water in soils at different moisture contents is of considerable theoretical interest as well as of practical importance, and in view of the fact that the mathematical consideration of the experimental data by Keen indicate conclusions the opposite from those published by the writer, it was decided to reinvestigate experimentally the whole problem more extensively and thoroughly than it had been done before. In the following pages, therefore, are presented the experimental results obtained and the conclusions to which they point.

AMOUNT OF UNFREE WATER IN SOILS AT DIFFERENT MOISTURE CONTENTS

The method employed for ascertaining whether the amount of water rendered unfree by the soil varies with the different moisture contents or remains constant, was the dilatometer method.¹ The procedure consisted of placing soil of different moisture contents in the dilatometer and determining the amount of water that refused to freeze at different temperatures. Two different temperatures or supercoolings were employed: -1.5° and -4°C . The object of employing these two temperatures was to obtain data both for the total unfree water and for the combined water. As previously stated all the water that refuses to freeze for the first time at the supercooling of -1.5°C . is designated as unfree water and is composed of capillary-adsorbed and of combined water. The capillary-adsorbed water will finally freeze at the temperature of -4°C ., but the combined will not even at the extreme low temperature of -78° .

By determining, therefore, both the total unfree and combined water the question as to whether the amount of water rendered unfree by the soil varies

¹ For detailed description of procedure and method see Bouyoucos (1).

with different moisture contents or remains constant, can be more conclusively answered.

In table 1 are presented the experimental results obtained. They show both the total unfree and combined water for a large number of soils at different moisture contents. Both the total unfree and combined water are expressed in percentages based only on the water added and on the air-dry soil. The water content of the soil represents only the water added and does not include the hygroscopic moisture. The hygroscopic moisture was not taken into consideration in the calculation of these results, first because it is desired to emphasize the fact that soils are able to cause water to become unfree even above the hygroscopic moisture, and second because from the practical standpoint it is the moisture above the hygroscopic point that is of importance.

From these results it becomes conclusively evident, therefore, that the amount of water which the soils are able to render unfree does not vary with different moisture contents but appears to remain constant. It will be readily seen that both the total unfree and combined water are practically the same at all the different moisture contents and in all the various classes of soil. There is a very slight tendency for both of these forms of water to be greater at the higher moisture contents, but this is largely due to the fact that at the higher moisture contents all the soil particles are more thoroughly and completely wetted and consequently the full amount of water is rendered unfree, and partly also to the fact that it is more difficult to expel all the air from the very wetted soils and this causes a slight error.

These experimental results, therefore, fully confirm the original suggestion made, namely, that the amount of water the soils cause to become unfree appears to be definite, and consequently they disagree with the conclusion derived mathematically by Keen. Evidently there are some unknown factors coming into play in the actual experimental results which are not taken into consideration in their mathematical treatment.

Although the total amount of unfree water seems not to vary at the different moisture contents it does appear, however, to vary with changes in the physical condition of the soil. As has already been stated the process of repeated freezing and thawing tends to liberate some of the unfree water and thereby diminishes considerably its original amount. Other treatments, such as coagulating or deflocculating the colloidal material in the soil by chemical agents, also may cause a variation in the total unfree water. The form of water which is susceptible to this change is the capillary-adsorbed. The combined does not appear to vary with physical changes in the soil, but seems to remain constant.

Although the free, capillary-adsorbed and combined water in the soil, as classified by the dilatometer method, exists in distinct forms, the transition from one into the other is not absolutely sharp. Some of the capillary-adsorbed water which is only weakly held by the forces of the soil is com-

TABLE 1

Amount of water rendered unfree by soils at different moisture contents, hygroscopic moisture not included

Coarse sand						
Moisture content, per cent.	5.00	7.50	10.00	12.50	15.00	75.00
Total unfree water, per cent.	1.21	1.52	1.60	1.42	1.51	1.62
Combined water, per cent.	0.82	0.68	0.92	0.63	0.84	1.03
Medium fine sand						
Moisture content, per cent.	5.00	7.50	10.00	12.50	15.00	75.00
Total unfree water, per cent.	1.84	1.98	1.57	1.83	1.93	2.16
Combined water, per cent.	1.25	1.37	1.18	1.45	1.43	1.51
Fine sandy loam						
Moisture content, per cent.	7.50	10.00	12.50	15.00	17.50	75.00
Total unfree water, per cent.	4.24	4.18	4.36	4.52	4.45	4.56
Combined water, per cent.	2.43	2.18	2.63	2.34	2.28	2.78
Loam						
Moisture content, per cent.	12.50	15.00	17.50	20.00	75.00	
Total unfree water, per cent.	8.56	8.17	8.73	8.38	8.82	
Combined water, per cent.	4.51	4.35	4.16	4.72	4.94	
Silt loam						
Moisture content, per cent.	12.50	15.00	17.50	20.00	75.00	
Total unfree water, per cent.	9.75	9.35	9.52	9.81	9.84	
Combined water, per cent.	5.28	5.42	5.37	5.61	5.45	
Silty clay loam						
Moisture content, per cent.	15.00	17.50	20.00	22.50	75.00	
Total unfree water, per cent.	13.62	14.03	13.85	14.04	14.25	
Combined water, per cent.	7.54	7.61	7.28	7.73	7.92	
Clay loam						
Moisture content, per cent.	15.00	17.50	20.00	22.50	75.00	
Total unfree water, per cent.	13.13	12.85	12.87	13.24	13.61	
Combined water, per cent.	7.28	7.42	7.17	7.04	7.58	
Humus clay loam						
Moisture content, per cent.	17.50	20.00	22.50	25.00	75.00	
Total unfree water, per cent.	15.35	15.82	15.18	15.61	16.34	
Combined water, per cent.	8.73	8.22	9.05	8.82	9.18	
Clay						
Moisture content, per cent.	20.00	22.50	25.00	27.50	75.00	
Total unfree water, per cent.	14.65	15.18	14.91	15.61	15.45	
Combined water, per cent.	9.08	9.23	9.35	9.45	9.68	
Clay						
Moisture content, per cent.	20.00	22.50	25.00	27.50	75.00	
Total unfree water, per cent.	15.24	15.46	15.18	15.62	15.85	
Combined water, per cent.	10.54	10.65	10.56	10.81	11.18	
Muck						
Moisture content, per cent.	80.00	100.00	120.00	140.00	200.00	
Total unfree water, per cent.	63.82	64.15	65.16	64.23	65.28	
Combined water, per cent.	42.53	42.35	42.91	43.28	44.19	
Peat						
Moisture content, per cent.	80.00	100.00	120.00	140.00	200.00	
Total unfree water, per cent.	69.23	70.15	69.18	70.35	71.13	
Combined water, per cent.	51.28	51.93	52.18	52.62	53.95	

pelled to freeze as free water by the force of freezing or crystallizing. A small part of the capillary-adsorbed water which exists in the most intimate contact with the soil and is held with the greatest force, probably does not freeze. Hence, the curves showing the rate of evaporation of water, and the freezing-point depression at different moisture contents, would not reveal any sudden breaks.

SUMMARY

In this paper are presented experimental results showing that the amount of water which the soils are able to render unfree does not vary with the different moisture contents but that it appears to remain constant.

This knowledge is of much importance, especially in relation to the availability of moisture in soils at different moisture contents, the possible behavior of soils toward fertilizers, the physical condition of the soil, etc.

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LIME REQUIREMENT AND REACTION OF LIME MATERIALS WITH SOIL

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The relative value of different lime materials has been determined in various ways. Large-scale field experiments conducted by a number of stations have afforded reliable information as to the effects upon crop yields to be expected after an application of the materials commonly used for liming. Numerous pot experiments have furnished indications of the effect of soil amendments never used in practice, but which are considered to be of interest from a theoretical point of view. The action of these materials has been explained from the effect upon the growth of particular crops, upon the formation of nitrates, upon the soil reaction as shown by various tests, and from the amounts of carbonate and total calcium and magnesium residual or found after intervals. In very few instances have the various proposed lime-requirement methods been considered sufficiently reliable to be of service as indicators of comparatively small changes brought about by the addition of basic materials to the soil.

The experimental work discussed in this paper was undertaken primarily to determine the adaptability of the vacuum lime-requirement method (2) to the measurement of differences in amounts of bases absorbed by soil. A secondary consideration was the comparison of the rates of reaction with soil, or relative "availability" of limestone and other basic materials. In a previous report (2) from this laboratory, it was stated that of the several methods tested, the vacuum method alone gave much promise, because it was satisfactorily quantitative in its indications and seemed to be universally applicable. Knight (7) made a comparison of results obtained by a lime-requirement method in which the hydrogen electrode was used, with those obtained in this laboratory by several methods, with the same soils. He found that the indications of his electrometric method and the vacuum method were substantially in agreement. Stephenson (12) has endorsed the principle of the method, i.e., the use of pure calcium carbonate and water only for measuring the soil's reactivity, but criticises the use of any heat whatsoever. On this phase of the subject, the present work has some bearing.

The comparative rates of reaction or absorption of bases by soil constituents from a high-calcium, nearly pure limestone in five different degrees of fineness have been determined. Calcite (Iceiand spar), magnesite and dolomite ground to pass a 100-mesh sieve, and the chemically prepared oxides and

carbonates of calcium and magnesium,¹ and two industrial slags,² in a comparable state of comminution, have been compared with the limestone. The rates of decomposition of finely divided limestone in one alkaline and two acid soils are compared. The conclusions reached have been based upon data obtained from carbonate determinations at stated intervals, and from lime-requirement determinations. In some instances, these data have been supplemented by determinations of accumulated nitrates.

The experiment here described was of a preliminary nature, and some of its features, in the light of later knowledge, were not perfect. Certain precautions in the matter of preparation of samples were shown by experience to be essential to the best results. The disturbing influence of nitrification was not sufficiently appreciated. At the same time, an account of the work may be of help to others, while the general results seem to be of sufficient interest and importance to warrant publication.

PLAN OF EXPERIMENT

A large amount of soil was obtained from the edge of one of the fertility experiment fields at the Ohio Agricultural Experiment Station. Although the soil was supposed never to have been limed, it is possible that it had at some time received a very light application when limestone intended for another part of the field was hauled over this part. The carbonate content of the soil is very small, but still distinctly larger than is found in the soil of the unlimed areas of the station farm. Otherwise, it was typical Wooster silt loam, acid to litmus paper and the Truog test (14). The surface soil only, to the depth of 7 inches, was taken. After partial drying, it was sifted through a screen of 2-mm. mesh, all the coarser material being rejected. Single pots also were prepared, one with an acid clay loam soil of the Trumbull series from one of the station's experimental fields at Strongsville, the other with an alkaline silty clay loam of the Clyde series, from the Paulding County experimental farm. These latter soils had been in storage for a year or more and were air-dry. Both were sifted in the same manner as the Wooster soil.

¹ The magnesium compounds were the U. S. P. "light" variety.

² The material designated "Slag (Al)" was supplied by the Electric Smelting and Aluminum Co., Sewaren, N. J. It is the *dicalcium silicate* discussed by Hartwell and Pember (6), in relation to its influence upon plant growth. "Slag (B.F.)" is blast furnace slag. The analyses of these slags were stated by the manufacturers to be as follows:

	Slag (Al)	Slag (B.F.)
SiO ₂	28.7	31.6
Al ₂ O ₃	6.3	14.9
Fe ₂ O ₃	1.9	—
CaO.....	46.8	36.9
MgO.....	2.9	15.2
Na ₂ O.....	6.5	—
CO ₂ and H ₂ O.....	6.9	—
S.....	—	1.8

Unless otherwise stated, the materials to be mixed with the soil were ground to pass through a sieve having 100 meshes to the linear inch. They were added at the rate of 0.310 per cent pure calcium carbonate in the air-dry mixture in the case of the pots with Wooster soil and 0.304 per cent pure calcium carbonate in the air-dry mixtures of Strongsville and Paulding soils. The actual amounts of the several amendments to furnish bases equivalent to the specified percentages of calcium carbonate were determined by titration in a manner similar to that described by Conner (4), except in case of the slags.

These were applied at such a rate as to carry calcium and magnesium equivalent to the other applications. It should be noted, however, that both slags were almost entirely soluble in normal hydrochloric acid.

Portions of soil of 11.34 kgm. each were weighed into a large container, then transferred to another, a little at a time, the lime material being gradually added and thoroughly worked in with the hands to avoid lumps. The mixture was then poured back and forth ten times, passing through a screen of 1-cm. mesh each time, and finally placed in a stoneware crock 20 cm. in height by 25 cm. in diameter. Here it was thoroughly compacted and 2 liters of distilled water added. An attempt to simulate natural conditions was made, although there was no crop on the pots. To this end, the pots were neither excessively watered nor permitted to dry out to an extent which would have caused severe injury to a crop. There was no drainage from the pots. They were kept outside from August 27 to November 10, the rainfall being supplemented by water as necessary. From November 10 to March 14 the pots were in a heated greenhouse, and were given 2 liters of water each week.

SAMPLING AND ANALYTICAL METHODS

Samples were taken from some or all of the pots on September 12, September 24, November 10 and March 14. The samples were taken by means of a steel sampling tube, of 16.5 mm. internal diameter, driven to the bottom of the pot. About five tubes full were taken at each sampling, and the holes were not closed. The samples taken September 12 were not dried, but were mixed as well as possible and portions for analysis weighed out at once. Subsequent samplings were air-dried as rapidly as possible and ground $\frac{1}{2}$ hour in a porcelain jar mill with flint pebbles.

Residual carbonate was determined in 20-gm. samples by the modified Marr method, (10) titrating the residual $\text{Ba}(\text{OH})_2$. The usual 1 to 50 HCl was employed for the decomposition of all samples except those treated with magnesite, for which 1 to 10 HCl and boiling at atmospheric pressure were necessary. A blank on untreated soil was always run with these.

The lime-requirement determinations by the vacuum method were made in the same apparatus as was used for carbonate determinations; 20-gm. portions of the soil were boiled *in vacuo* for $2\frac{1}{2}$ hours with 80 to 100 cc. of water and 2 gm. of chemically pure precipitated calcium carbonate, the carbon

dioxide evolved being estimated as in the carbonate determinations. The temperature of the boiling mixture was very near 50°C. unless otherwise stated, and in the cases of samples taken at the two later dates, the temperature was accurately controlled by adjusting the pressure within the apparatus according to the indications of a thermometer hung in the lower part of the condenser. A number of determinations made at temperatures lower and higher than 50° were made and will be discussed.

For the few nitrate determinations made, the soil was extracted with water and the phenoldisulfonic acid colorimetric method employed (11).

SAMPLES TAKEN AT THE END OF 16 DAYS

The pots containing Wooster soil with limestone finer than 100-mesh and Paulding soil with limestone were sampled September 12. These samples were not dried, but were thoroughly mixed, and the analytical work done at once. The moisture contents were determined and the results are calculated to the air-dry basis, so as to be comparable with later data. The carbonate content of the original Wooster soil, unground and at 3.7 per cent moisture content as when put in the pots, was 0.039 per cent CaCO_3 on the air-dry basis, or 2.0 per cent moisture. The lime requirement on the same sample was 0.214 per cent CaCO_3 . The sample from pot 1, Wooster soil plus 0.310 per cent CaCO_3 in carbon limestone past 100-mesh, showed residual carbonate equivalent to 0.208 per cent CaCO_3 on the air-dry basis, and the lime requirement had been reduced to 0.075 per cent CaCO_3 . Taking into account the carbonate content of the soil at the beginning, we have 0.349 less 0.208, or 0.141 per cent CaCO_3 disappeared. The lime-requirement data indicate a reduction in lime requirement from 0.214 to 0.075, or 0.139 per cent CaCO_3 .

The original air-dry but unground Paulding soil showed a carbonate content of 0.010 and a lime requirement of 0.224 per cent CaCO_3 . The sample from pot 17, Paulding soil with an addition of 0.304 per cent CaCO_3 , supplied by limestone past 100-mesh, showed residual carbonate equal to 0.179 per cent, and residual lime requirement of 0.102 per cent CaCO_3 . From this soil, 0.135 per cent CaCO_3 had disappeared, but the lime requirement had been reduced only 0.122 per cent CaCO_3 . From the acid Wooster soil, 42 per cent and from the alkaline Paulding soil 46 per cent of the added carbonate disappeared during the period of 16 days.

SAMPLES TAKEN AT THE END OF 27 DAYS

The samples taken September 24, about a month after the experiment had been begun, included all the pots except those treated with the coarser limestone. These and subsequent samples were air-dried, and ground previous to analysis. The data are presented in table 1. In the column headed "added bases remaining as CaCO_3 ," it will be noted that the residual carbonate had decreased in the case of the Wooster soil with limestone (pot 1)

from 58 to 50 per cent of the amount added. With the Paulding soil (pot 17) the percentages were 54 and 47. The Strongsville soil (pot 16), first sampled at this time, showed only 14 per cent remaining of the same addition of limestone as was made in the case of the Paulding soil.

Calcite and magnesite (pots 6 and 7) were apparently slightly more resistant than limestone to the agencies of decomposition in the Wooster soil. Dolomite (pot 8) was considerably more resistant, as has been noted by Stewart and Wyatt (13). Precipitated calcium carbonate (pot 9) was much more easily attacked than the natural carbonates, only 39 per cent remaining, while 17 per cent of an equivalent amount of base added to pot 10 in the form of calcium oxide was present as carbonate. Magnesium oxide (pot 11) was apparently slightly less reactive than calcium oxide; less carbonate was residual, but the magnesia had not reduced the lime requirement to the same extent. Of the magnesium carbonate added to pot 12, none remained in the carbonate form. The ready decomposition of this material when mixed with soil has been noted by MacIntire (8); a greater reduction of lime requirement, however, was caused by magnesium oxide and the calcium compounds. The slag (Al), "dicalcium silicate," added to pot 13 was apparently quite active. A part of its base content seems to have changed into carbonate, since the amount of carbonate found in the soil receiving this treatment was greater than was originally present and added in the material, while the lime requirement was reduced by a figure equal to that shown where precipitated calcium carbonate was the treatment. The blast furnace slag added to pot 14 was apparently less active than any of the other materials.

The column headed "bases not found as CaCO_3 " refers to a percentage of calcium carbonate, calculated on the basis of air-dry soil, equivalent to the bases added in any form but not residual as carbonates. In the case of carbonated materials, this figure would represent the material which has reacted with soil constituents, but with materials other than carbonates it could possibly represent material lying inert and unchanged, as well as that which has been acted upon. The column headed " CaCO_3 requirement reduction" contains the percentages of calcium carbonate, referred to the air-dry soils, corresponding to the difference between the lime-requirement data for the untreated soil and that of each differently treated soil. These figures for lime-requirement reduction should correspond to the differences between the bases added and the carbonate residual in the cases of those pots treated with carbonate materials, provided that there is no base consumed by biological processes such as nitrification or by reaction with soil constituents which do not exhibit their full absorptive capacity during the lime-requirement determination.

In the cases of all the pots of Wooster soil treated with natural carbonate and sampled at this time, the correspondence is quite close, and would indicate that the vacuum lime-requirement method affords indications which are quite satisfactory for purposes of comparison, at least. The Strongsville and

Paulling soils treated with limestone exhibit considerable discrepancies in this respect, however, as likewise do those pots of Wooster soil treated with the more active precipitated carbonates or oxides.

SAMPLES TAKEN AT THE END OF 75 AND 199 DAYS

The samples taken November 10 and March 14 will be discussed together. The natural carbonates have been further decomposed, and their relative resistance to decomposition does not differ materially from that indicated at the earlier sampling. The precipitated calcium carbonate has undergone further decomposition, although at the last sampling more carbonate was found in the soil from the pot treated with this material than was found at the earlier date, a circumstance difficult to account for. The second sampling indicates a slight increase in carbonate content for pot 10, treated with calcium oxide, but at the third sampling it is at the same figure as at first. The increase in carbonate content noted at the second sampling might be due to the carbonation of calcium oxide previously unaffected, although the findings of MacIntire are rather against this explanation. This investigator (9) concludes that if conditions are at all favorable to carbonation or reaction, caustic or hydrated lime does not long persist in the soil. A statement to the same effect has been attributed to Hager (5), although the writer has not seen the original article. The magnesium-oxide and carbonate treatments both appear to have resulted in a later formation of carbonate, although this is inconsiderable in the case of the magnesium carbonate.

The three soils treated with limestone past 100-mesh show in these last samples about the same relative amounts of residual carbonate as were found in the earlier samples. It seems rather remarkable that at the end of six months limestone should survive nearly as well in a soil acid to litmus paper as in one alkaline to that test, while in the acid Strongsville soil the limestone has practically disappeared at the last sampling.

The four pots of Wooster soil treated with limestone coarser than 100-mesh were sampled at the end of 75 and 199 days only. The results for carbonate content indicate that the distribution of the coarser particles of limestone through the soil in the pots was very imperfect. The indications of the lime-requirement determinations seem more reliable, although some of these are not entirely consistent. At any rate, these coarser materials are indicated to be much less active than the same stone when more finely divided, which agrees with the conclusions of several investigators, based upon evidence along other lines.

CHANGES IN LIME-REQUIREMENT REDUCTION

Upon reference to the data for CaCO_3 -requirement reduction, assembled in table 1, it will be noted that the later samplings show a progressive increase in the lime requirement satisfied, in the case of the pots treated with the

TABLE 1
Changes in lime materials and reduction of lime requirement

POT	TREATMENT	28 DAYS			75 DAYS			199 DAYS		
		Added bases remaining as CaCO_3	Bases not found as CaCO_3	CaCO_3 requirement reduction	Added bases remaining as CaCO_3	Bases not found as CaCO_3	CaCO_3 requirement reduction	Added bases remaining as CaCO_3	Bases not found as CaCO_3	CaCO_3 requirement reduction
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Wooster 1	Limestone, past 100-mesh	50	0.156	0.164	48	0.161	0.157	38	0.192	0.176
Wooster 2	Limestone, 20- to 40-mesh				84	0.051	0.030	49	0.159	0.028
Wooster 3	Limestone, 40- to 60-mesh				108		0.049	76	0.075	0.082
Wooster 4	Limestone, 60- to 80-mesh				86	0.044	0.087	60	0.123	0.129
Wooster 5	Limestone, 80- to 100-mesh				74	0.080	0.091	61	0.120	0.110
Wooster 6	Calcite, past 100-mesh	53	0.147	0.145	47	0.163	0.157	36	0.200	0.191
Wooster 7	Magnesite, past 100-mesh	54	0.113	0.111	54	0.142	0.120	33	0.207	0.154
Wooster 8	Dolomite, past 100-mesh	73	0.084	0.097	71	0.091	0.101	55	0.140	0.119
Wooster 9	CaCO_3 , precipitated	39	0.190	0.173	19	0.252	0.180	26	0.229	0.173
Wooster 10	CaO , past 100-mesh	17	0.257	0.207	20	0.248	0.189	17	0.257	0.176
Wooster 11	MgO , past 100-mesh	11	0.276	0.198	14	0.267	0.166	16	0.262	0.120
Wooster 12	MgCO_3 , past 100-mesh		0.310	0.164	1	0.306	0.124	3	0.301	0.124
Wooster 13	Slag (Al), 100-mesh	25	0.231	0.173	29	0.219	0.166	18	0.253	0.174
Wooster 14	Slag (B.F.), 100-mesh	1	0.306	0.079	4	0.296	0.045	2	0.305	0.059
Wooster 15	Check									
Strongsville 16	Limestone, past 100-mesh	14	0.261	0.284	9	0.275	0.304	5	0.290	0.270
Paulding 17	Limestone, past 100-mesh	47	0.161	0.116	44	0.170	0.127	37	0.188	0.151

natural carbonates, possibly excepting the Strongsville soil. Those pots which received the artificially prepared amendments, however, show in a greater or less degree exactly the opposite effect. This suggests that nitrification has been very active in some of the pots, and accordingly the later samples from some of the pots were examined for nitrate content. The amounts of nitric nitrogen found, calculated to the equivalent percentage of calcium carbonate required for neutralization, are shown in table 2. The nitrate content of the original soils is not included, because it was found to be so small as to be quite negligible.

TABLE 2
Bases consumed in nitrification

POT	CALCIUM CARBONATE EQUIVALENT TO NITRATE	
	At the end of 75 days	At the end of 199 days
	<i>per cent</i>	<i>per cent</i>
1	0.014	
2	0.008	
3	0.009	0.032
4	0.009	0.046
5	0.014	0.060
6	0.012	
7		0.016
8		0.012
9	0.017	0.038
10	0.023	0.011
11		0.054
12		0.054
13	0.024	0.040
14	0.011	0.033
15	0.006	
16	0.023	0.071
17	0.038	0.033

The data in table 2 show that the chemically prepared oxide and carbonate of calcium and magnesium and precipitated calcium carbonate are quite active in promoting nitrification. The quantities of nitrate produced to be sure are insufficient to account for all the change in lime requirement observed in the cases of the magnesium compounds, but these must be considered to be minimum figures for nitrates. The repeated wetting of the soil in the pots would inevitably lead to the concentration of the nitrates in those portions of the soil most exposed to the air, and from which evaporation must take place. The presence of unfilled holes from former samplings would result in such a concentration in the soil immediately adjacent, and as later samples were always taken from a quarter of the pot not previously sampled, a fair share of the nitrate content was probably not secured. Besides, as Allen and Bonazzi (1) have shown, a simple water extraction, as was here used, is not sufficient for the quantitative determination of nitric nitrogen in soil.

MANNER OF PREPARING SAMPLE, TEMPERATURE AND LIME-REQUIREMENT INDICATIONS

A common ground for criticism of lime-requirement methods is that their indications are affected by comparatively slight variations in conditions. The procedure used in this investigation is no exception to the general rule. The following figures in table 3 will illustrate the point. The soil is the Wooster silt loam used for these pots, one sample being taken from the untreated check, pot 15, the other from pot 1, treated with limestone past 100-mesh equivalent to 0.310 per cent CaCO_3 . The soil contained originally 0.029 per cent CaCO_3 , making the carbonate content at the beginning of the experiment 0.339 per cent CaCO_3 . At the end of 75 days, the time at which the samples were taken, there was found 0.178 per cent CaCO_3 , indicating that 0.161 per cent CaCO_3 had reacted with soil constituents. The differences in lime requirement, as determined by a procedure uniform except in respect to the degree of heat employed, are seen to vary with the temperature at which the determination was made. At temperatures above 40°C ., the difference appears to be constant at about 0.168 per cent CaCO_3 . The amount of nitrate found indicated that at least 0.014 per cent CaCO_3 was consumed in its formation, more than sufficient to account for the excess of carbonate disappeared, even after considering the nitrate found in the soil from the check pot.

It will be recalled that there were considerable discrepancies between the amounts of base not appearing as carbonate and reductions in lime requirement in the case of pot 10, Wooster soil treated with calcium oxide. The lime-requirement data for soil from this pot, sampled at the end of 9 weeks, and determined at three temperatures, are included in table 3. The comparison in this case is between the sample from pot 10 and the original Wooster soil, the sample from the check, pot 15, having been exhausted. However, as may be seen from data for determinations at 33° and 50°C ., the original sample and that from pot 15 give nearly the same values for lime requirement, when run at the lower temperatures. The data in table 3 demonstrate very plainly that the figures for lime requirement are too small except at the highest temperature, because carbonate determinations showed that the soil had absorbed the equivalent of 0.248 per cent CaCO_3 , more than the indicated lime requirement at the lower temperatures. A certain amount of this base absorption was doubtless due to nitrification, the equivalent of 0.023 per cent CaCO_3 being found in the sample under consideration. If this nitrate content is considered, the reduction in lime requirement is found to be greater than the consumption of base, exclusive of that required for nitrate formation.

This discrepancy is not great, and might well be ascribed to analytical error, but as will shortly appear, a similar and even larger difference is noted in the cases of the Strongsville and Paulding soils. The explanation is simple and since it will direct attention to certain precautions necessary in studies of this kind, will be given in detail. As was stated, the samples from pot 10 dis-

cussed in table 3 are compared with the sample of Wooster soil taken at the beginning of the experiment and not with samples from the check, pot 15, since the supply from the latter was exhausted. There were no check pots for the Strongsville and Paulding soils, the comparisons in these cases being in all cases with the samples taken at the beginning. These samples were large, and to save time were ground in a large porcelain-jar mill with flint pebbles, while the smaller samples taken from pots were ground in a small mill of the same design. After grinding the samples, it was noticed that the larger jar was considerably more efficient than the smaller one, and the samples ground in it were finer, although the time of grinding had been the same. The effect of very fine grinding of the sample upon the indications of the Veitch lime-requirement method has been noted by Brown and Johnson (3). The effect as measured by the procedure used in this work is in the opposite direction, that is, finer grinding causes an increase in lime requirement instead of a decrease as with the Veitch method. The following will serve to illustrate the influence of fineness of sample upon the indications of the vacuum method, other conditions being practically the same. The soil is that used for these pots, the temperature about 50°C. The unground soil showed a lime requirement of 0.218 per cent CaCO_3 , that ground by hand in an agate mortar 0.260 per cent, and that ground in the porcelain jar with flint pebbles for about an hour, 0.302 per cent.

The Strongsville soil, pot 16, treated with 0.304 per cent CaCO_3 in limestone past 100-mesh, shows with increases in temperature the same increasing difference in lime-requirement indications when compared with the original soil. Carbonate determinations indicate that 0.275 per cent CaCO_3 has been consumed, but the lime-requirement determinations at 50°C. indicate a difference of 0.304 per cent and at 100°C. a difference of 0.351 per cent CaCO_3 . This soil has a high lime requirement and shows more plainly than the Wooster soil the effect of differences in the manner of preparation.

The Paulding soil, pot 17, treated with 0.304 per cent CaCO_3 in limestone past 100-mesh, exhibits differences in lime-requirement indications smaller than those shown by the other soils considered. Carbonate determinations indicated the disappearance of 0.170 per cent CaCO_3 , and nitrification would account for at least 0.038 per cent CaCO_3 , leaving 0.132 per cent to represent the base-satisfying lime requirement. As in the other cases, the difference between the lime-requirement indications obtained from determinations at 100°C. considerably exceeds the probable figure, while at 33°C. there is a deficiency. The excessive figures obtained at the higher temperature are probable due to the difference in the degree of fineness of the samples, while the deficiency at the lower temperature can be attributed only to an incomplete reaction.

TIME ALLOWED FOR REACTION, AND LIME-REQUIREMENT INDICATIONS

The fact that with a fixed time, an increase in temperature causes the difference in lime requirement between the treated and the untreated soils to approach the probable figure for base absorbed, suggests that with a fixed temperature, an increase in time allowed might show the same result.

The Wooster soil, sampled at the beginning of the experiment, showed a lime requirement of 0.283 per cent CaCO_3 , the temperature being 50°C . and the time $2\frac{1}{2}$ hours. At the end of the period stated, the absorption apparatus only was disconnected and replaced with one freshly filled, the apparatus

TABLE 3
CaCO₃-requirement indications at various temperatures

	30°C.	33°C.	40°C.	50°C.	60°C.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Pot 15, check	0.220	0.240	0.262	0.293	0.304
Pot 1, limestone	0.084	0.094	0.094	0.126	0.136
Difference	0.136	0.146	0.168	0.167	0.168

	33°C.	50°C.	100°C.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Original Wooster soil	0.225	0.283	0.552
Pot 10, Wooster soil plus CaO	0.061	0.094	0.305
Difference	0.164	0.189	0.247
Original Strongsville soil	0.454	0.571	1.006
Pot 16, Strongsville soil plus limestone	0.220	0.267	0.655
Difference	0.234	0.304	0.351
Original Paulding soil	0.271	0.337	0.707
Pot 17, Paulding soil plus limestone	0.164	0.210	0.537
Difference	0.107	0.127	0.170

again evacuated and the experiment continued as before. At the end of the second $2\frac{1}{2}$ hours, the lime requirement was found to be 0.023 per cent CaCO_3 additional, or 8 per cent of the original indication. A sample from pot 1, treated with calcium carbonate, showed 0.126 per cent and 0.019 per cent, respectively. With these soils, the additional time makes a scarcely noticeable difference in the final result.

Under the same conditions, the Strongsville soil showed in the first $2\frac{1}{2}$ hours 0.570 per cent, in the second 3 hours, 0.075 per cent and in the third $1\frac{1}{2}$ hours, 0.023 per cent CaCO_3 required. With the Paulding soil run at the same time, the figures were 0.337, 0.037, and 0.025 per cent CaCO_3 required. In each case the first indication is very nearly 85 per cent of the sum of the three. There are no figures corresponding to these for the Paulding and Strongsville soils treated with limestone, but if it is assumed that the same percentage

relation between the lime requirement in $2\frac{1}{2}$ hours and that found in the longer period would exist with the limed soil, the 0.210 per cent CaCO_3 required in the case of the limed Paulding soil run $2\frac{1}{2}$ hours would become 0.247 per cent. The difference between this and the sum of the several requirements of the unlimed soil is 0.152 per cent, or 0.020 per cent in excess of the base actually consumed less that corresponding to the nitrates found. A similar calculation with the data from the Strongsville samples indicates a difference in lime requirement of 0.354 per cent CaCO_3 , considerably more than was actually added. These excessive indications are probably due to the difference in the degree of fineness of the samples, as has been explained. In spite of this factor, also applying to the comparison of soil from pot 10, Wooster soil, with calcium oxide equivalent to 0.310 per cent CaCO_3 , with the original sample of Wooster soil, the assumption that the lime-requirement indication obtained in $2\frac{1}{2}$ hours at 50°C . is 85 per cent of the maximum obtainable by long-continued action at that temperature, appears to afford correct indications. The difference in the calculated lime requirements is 0.222 per cent CaCO_3 ; the base absorbed less the nitrate found is equal to 0.225 per cent CaCO_3 .

The data indicate that at the moderate temperature of 50°C . the reaction between the soils tested and calcium carbonate is sufficiently complete in 6 or 8 hours for differences to be practically quantitative. At lower temperatures a longer time would probably be required, at higher temperatures the time of reaction might be shorter. There does not appear to be any reason for the belief that any procedure of aeration with agitation would shorten the time; in the 200-cc. globular flasks used, the agitation accompanying boiling *in vacuo* is very thorough, while no more efficient procedure for the removal of CO_2 can be devised.

SOIL ACIDITY AND LIME REQUIREMENT

It has been stated that the Wooster and Strongsville soils are acid to litmus paper and the Truog test (14), while the Paulding soil is alkaline to litmus paper and the Veitch test (15) and gives only a very faint reaction for acidity by the Truog test. Water extracts of the original soils, air-dried and ground, were prepared in the proportion of 50 gm. of soil to 100 cc. of water, shaken occasionally for several hours and filtered through paper.

The clear filtrates were carefully compared as to color developed with various indicators, according to the plan of Wherry (16). It was easily seen that the extracts differed in reaction toward certain indicators, the Strongsville soil giving a strongly acid extract, the Wooster soil one distinctly acid, while that from the Paulding soil was distinctly alkaline. The pH values, as nearly as could be determined from the colors developed with the indicators, without any comparison with standard buffer mixtures, were within limits as follows: Wooster soil, pH 6.0 to 6.5, Strongsville soil, pH 5.5 to 6.0, Paulding soil, pH 7.0 to 7.5.

The Strongsville soil is most strongly acid, the reaction between it and limestone has been most rapid, and at the same time it has the highest lime requirement. These facts all agree with what might be expected, but a comparison of the Wooster and Paulding samples brings out some surprising features. Limestone disappears at nearly the same rate from the alkaline Paulding soil as from the acid Wooster soil, base consumed in nitrification being considered. The lime requirement of the Paulding soil is about the same as that of the Wooster soil. From this, it would seem to follow that the total amount of unsatisfied base-absorptive capacity has perhaps as much influence upon the rate of reaction between the soil and lime materials as has the intensity of acidity.

Soil acidity and lime requirement are not synonymous terms, as some have assumed them to be. A soil may have a considerable lime requirement, but may not be acid nor in need of liming. There are certainly active base absorbents unsatisfied in neutral soils—an observation by Winter (17) demonstrates this very plainly. And it has been shown that a soil, although giving an alkaline water extract, is still capable of decomposing calcium carbonate at ordinary temperatures. In view of these facts, it would seem that the idea that lime requirement is a measure of the soil acidity to be corrected by liming is in need of revision.

That a laboratory procedure which will give an accurate indication of the optimum application of lime to the soil under field conditions would be very desirable is an obvious fact. The number of methods which have been proposed for the purpose is evidence of the attention soil chemists have devoted to the subject. All of these methods are more or less arbitrary and empirical. This is not written in a spirit of criticism; any analytical procedure applied to soil, the final criterion of its value being the behavior of crops upon that soil, cannot well be otherwise in its earlier stages. From a consideration of the numerous and varied factors which govern the utilization of basic materials in the soil and the loss of bases therefrom, it would seem that no laboratory method can reasonably be expected to give more than a very roughly approximate indication of the most profitable amount of lime to apply.

It is the writer's opinion that the perfect lime-requirement method, or perhaps a better expression would be, the perfect method for the determination of base absorption, will not find its greatest usefulness in the field mentioned. Rather, it will be a measure of changes in the soil's capacity for absorption of bases, with particular reference to calcium, as the result of liming, cropping, etc. It will scarcely be expected to be any more of a guide for field treatment than the determination of total calcium might be, yet by its means, comparatively slight changes in the relations between added bases and the soil may be followed. It should be quantitative in the sense that differences are indicated with great precision, although perhaps not measuring the total capacity of the soil for reaction with bases.

The results obtained in the present investigation appear to indicate that the reaction between soil and precipitated calcium carbonate can be made quantitative to a sufficient degree to serve as the basis for a satisfactory lime-requirement method. In order to attain this end, however, it will be necessary to devote great care to the preparation of samples and all details of the actual determination must be as uniform as possible. The soil should be finely ground; a porcelain-jar mill with flint pebbles is a satisfactory means for grinding, and it would probably not be too extreme a precaution to use the same mill and pebbles for all samples to be compared, and to charge it with the same weight of soil, as well as to have the speed and time of grinding equal in all cases. Finally, it is emphasized that the indications obtained have their chief value from a comparative standpoint, and it does not matter what the absolute figures are, if differences between samples of the same soil differently treated are indicated with accuracy. Hence, any of the methods of procedure suggested, such as aspiration with agitation at room temperature or boiling at a definite temperature *in vacuo* or at atmospheric pressure, ought to be equally satisfactory, provided only that such procedure is continued for a sufficient length of time for the reaction between the soil and calcium carbonate to afford a practically quantitative indication of differences in base-absorbent capacity.

SUMMARY

Data from an experiment in which various lime materials were mixed with soil in undrained pots, watered and kept partly outside and partly under greenhouse conditions, are presented in this paper. The conclusions formed are based upon determinations of residual carbonate, lime requirement and accumulated nitrates.

A relatively pure high-calcium limestone, calcite (Iceland spar) and magnesite were very similar in behavior. Approximately one-half of an application equivalent to 3.1 tons per acre of these materials ground to pass a 100-mesh sieve had been attacked within 4 weeks, and two-thirds within 28 weeks. The natural carbonate dolomite was found to be about 50 per cent more resistant than the other materials, while precipitated calcium carbonate was much more easily attacked by the agencies of decomposition.

Caustic lime is apparently more reactive than the precipitated carbonate, although with time the differences noted become less. Carbonate formation was found to result from an application of chemically prepared magnesium oxide, and the amounts found gradually increased with time. On the other hand, the disappearance of carbonate from an equivalent application of chemically prepared magnesium carbonate was complete within a month, but traces of carbonate were afterwards formed.

Of two samples of slag tested, "dicalcium silicate" was found to react with soil as readily as precipitated calcium carbonate, but blast-furnace slag was less reactive than any other material tested.

Coarsely ground limestone had much less effect in reducing lime requirement than the finely ground.

Limestone ground to pass a 100-mesh sieve was found to be utilized at nearly the same rate in an acid silt loam and an alkaline clay loam, but carbonate disappeared much more rapidly from an acid clay loam. The lime requirements by the vacuum method were comparable in the cases of the first two soils, but twice as great with the third.

The quantitative relations between bases not residual as carbonate and reductions in lime requirement were found to be reasonably close, a possible consumption of bases by nitrification being considered. The evidence obtained in the present investigation indicates that the interaction of soil constituents and calcium carbonate is sufficiently regular and quantitative for the indications of a lime-requirement method based upon the reaction, to be of practical utility, provided the proper precautions are observed.

The indications of the lime-requirement method employed were found to depend to a great extent upon such factors as the manner of preparation of the sample, the temperature and the time allowed for the determination.

No conclusive evidence has been obtained that heating is an undesirable feature of a perfect lime-requirement method, if we understand by that term a satisfactory method for the study of the relations between base absorbents and added bases in the soil. On the other hand, heat may be of great advantage in hastening a naturally slow reaction and may enable differences to be indicated with an exactness not otherwise attainable in a reasonable length of time.

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EFFECT OF SALT SOLUTIONS HAVING DEFINITE OSMOTIC CONCENTRATION VALUES UPON¹ ABSORPTION BY SEEDS¹

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INTRODUCTION

The purpose of this paper is to report a part of the data obtained from a series of experiments which were designed to determine to what extent salt solutions with definite osmotic concentration values tend to resist water absorption by seeds placed in contact with them, and whether this influence has a direct bearing upon germination. The data here presented deal only with that portion of the work which has to do with the rates of imbibition as these are influenced by the osmotic concentrations of the different solutions employed.

It is, of course, generally understood that salts in solution offer resistance to water absorption by plant roots in contact with them because of the osmotic properties of the solutions, and it appears that these same physical properties influence in a somewhat similar manner the imbibition of water by seeds. Absorption from salt solutions by seeds has been studied by a number of investigators under different conditions and the results obtained are somewhat conflicting. No attempt can here be made to review the literature on imbibition by seeds and its relation to germination, but a few of the more definite contributions on this subject may be mentioned as having a direct bearing on the present study.

Slosson (5) working with a large number of different kinds of seeds has shown that the presence of salts in solution hinders the absorption of water. This same author points out that retardation of imbibition does not increase proportionately with the osmotic pressure of the solutions but less rapidly, and that isosmotic solutions produce practically the same effect, showing that osmotic pressure is of greater importance than the kind of salts.

In order to decide whether the absorption of water by seeds is physical or physiological, Buffum (2), investigating the effect of salt concentration on the germination of seeds of different species, came to the conclusion that the retarding effect of a salt solution on germination is in direct proportion to the osmotic pressure when the solutions are strong.

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Slosson and Buffum (6) made comparative tests between living and dead seeds in which the power of germination had been destroyed by age, heat, or exposure to formaldehyde vapor. They came to the conclusion that there is no difference between living and dead seeds with respect to the absorption of water, and that water is drawn into the seeds by purely physical forces such as surface tension and osmotic pressure.

Atkins (1) made an attempt to discover the forces which cause water and salt solutions to enter dried seeds. Seeds were immersed in water and in various salt solutions. Changes in the concentration of the salt solutions were determined by volumetric methods and the conclusion drawn is that "the rate at which distilled water is taken up is no greater than that at which salt solutions are absorbed," and that there is no difference in absorption between living and dead seeds until after germination. He states that the forces involved are capillarity and imbibition in the initial stages, and osmosis after germination.

Dachnowski (3), studying the effect of acid and alkaline solutions upon the water relation of plants, comes to the conclusion that the amount of water absorbed and retained by seeds in an acid solution is not dependent upon the concentration of the acid and is not a function of it. The addition to the solution of 0.00125 *N* HCl of a molecularly equivalent solution of any salt not reacting with the acid does not increase the quantity of water absorbed and retained by seeds of *Phaseolus*. However, a higher concentration of any salt is followed by an inhibition of the capacity for absorbing and retaining water. The conclusions reached for the results on absorption and retention of water by seeds in alkaline solutions are analogous to those for acids.

The work published by Shive (4) on the effect of salt concentration on the germination of seed in sand cultures clearly shows the retarding influence of solution concentration in a solid substratum upon water absorption by seeds. Single-salt solutions of definite osmotic concentration values were added to sand in the proportion of 100 cc. of solution to 1 kgm. of air-dry sand. Seeds of corn and beans were incubated in these cultures for periods of from 48 to 72 hours at room temperature. The author states that while germination was not prevented by the higher osmotic concentrations, it was considerably retarded, and that the retarded germination appeared to be directly related to the amounts of water imbibed by the seeds, which, in turn, is dependent upon the osmotic concentrations of the solutions.

The studies reported in the following pages, as already stated, deal simply with the absorption by seeds from salt solutions having definite osmotic concentration values and from distilled water. An effort was made also to determine the influence of time upon the rates of absorption by seeds from single-salt solutions and from a complete nutrient solution for plants.

METHODS

"Baker's analyzed" salts only were used. The salts used were $\text{Ca}(\text{NO}_3)_2$, K_2CO_3 , MgSO_4 , KH_2PO_4 , NaCl , KCl , NaNO_3 , and Shive's complete nutrient solution for plants, no. R_5C_2 . Half-molecular stock solutions of each salt were prepared in sufficient quantities to last throughout the experiment. From these stock solutions the more dilute solutions were prepared ranging in osmotic concentration values from 0.001 atmosphere to 7.0 atmospheres. The osmotic concentration values of these solutions were calculated to approximate the concentrations desired and were then tested by the freezing-point method and corrected whenever necessary. In table 1 are given the kind of salts, the volume-molecular concentrations, and the osmotic concentration values of the solutions employed. The solutions having osmotic concentration values below 0.5 atmosphere were calculated but not tested for

TABLE 1
Osmotic and volume-molecular concentrations of solutions used

OSMOTIC CONCENTRATION	VOLUME-MOLECULAR CONCENTRATIONS						
	MgSO_4	KNO_3	$\text{Ca}(\text{NO}_3)_2$	KCl	K_2CO_3	KH_2PO_4	NaCl
<i>atm.</i>							
0.5	0.0116	0.0098	0.0073	0.0099	0.0070	0.0105	0.0159
1.0	0.0232	0.0196	0.0146	0.0199	0.0144	0.0210	0.0319
2.0	0.0530	0.0417	0.0318	0.0398	0.0308	0.0420	0.0682
3.0	0.0814	0.0633	0.0495	0.0609	0.0466	0.0630	0.1032
4.0	0.1131	0.0876	0.0698	0.0839	0.0649	0.0840	0.1438
5.0	0.1416	0.1102	0.0896	0.1052	0.0826	0.1050	0.1829
6.0	0.1759	0.1326	0.1074	0.1305	0.0989	0.1260	0.2188
7.0	0.2060	0.1585	0.1297	0.1486	0.1193	0.1472	0.2639

correctness by the freezing-point method. The solutions comprised in a series increased in osmotic concentration from 1.0 to 7.0 atmospheres by equal increments of 1.0 atmosphere. The concentrations of the more dilute solutions in each series were 0.001, 0.01, 0.10, and 0.50 atmospheres.

The seeds employed comprised corn (*Zea mays*), soybeans (*Soja maxima*), alfalfa (*Medicago sativa*), white lupine (*Lupinus alba*), spring wheat (*Triticum vulgare*), watermelon (*Citrullus vulgare*), Canada field peas (*Pisum sativum*), Japanese buckwheat (*Fagopyrum esculentum*), and dwarf Essex rape (*Brassica napus*). Fifty seeds of the larger kind and 100 each of alfalfa and rape were weighed and placed in screw-cap bottles each containing 100 cc. of solution. To each series was added one control in distilled water for each species of seed. The bottles were placed on a laboratory table and the seeds were allowed to soak for a period of 15 hours, after which time the liquid was poured off and the seeds quickly placed between filter papers to remove the water on the surface of the seeds. The seeds were then placed in small bottles

to prevent evaporation during the time of weighing. The weights of the seeds were recorded and the per cent of solution absorbed and the relative absorption quantities were calculated, the control in distilled water being used as a unit.

EXPERIMENTAL WORK

I. The influence of osmotic concentration upon absorption by seeds

The influence of salt solutions of various osmotic concentrations is shown by the data recorded in table 5, which gives the osmotic concentration values of the solutions in atmospheres and the absorption amounts per gram of dry seed relative to the amount absorbed from distilled water taken as unity. The actual amount of absorption per gram of dry seed from distilled water is given in parenthesis, so that the corresponding amount of absorption from any

TABLE 2

Variation in the absorbing power of corn of the same variety grown under the same conditions but from ears of different plants; seeds soaked for a period of 15 hours in solutions of $\text{Ca}(\text{NO}_3)_2$

CONCENTRATION	ABSORPTION	
	Seeds from ears of plant 1	Seeds from ears of plant 2
<i>atm.</i>	<i>per cent</i>	<i>per cent</i>
7	31.2	28.0
6	31.1	27.6
5	31.5	29.4
4	32.0	29.0
3	31.8	29.8
2	32.6	30.2
1	32.1	30.0
0.5	34.7	32.4
0.10	35.2	32.7
0.01	34.8	32.4
0.001	35.3	32.7
0.00	35.6	32.9

solution may be obtained by multiplying the relative amount by the actual amount absorbed from distilled water as given in parenthesis in the same column. All experiments were made in duplicate if not otherwise stated. The data, therefore, represent the values obtained by averaging the corresponding data from the duplicate series. This was deemed necessary after the observation, made in the course of the study, that seeds of the same kind do not always absorb the same quantities of liquid per gram of dry seed, even though the osmotic concentration, temperature, and length of time exposed are the same. A striking example of this is given in table 2, which presents the data obtained with seeds of corn of the same variety and grown to maturity in the same field under approximately the same conditions, but taken from

ears of two different plants. The seeds were immersed in solutions of calcium nitrate for 15 hours, 50 seeds in each solution. Absorption by the seeds from the different plants, in general, followed the same order with respect to solution concentration, but the per cent of absorption throughout was higher for the seeds from one plant than it was for the seeds from the other, this difference approximating 2.5 per cent as an average for this series. In order to eliminate this factor of individual differences in absorption as far as possible, large quantities of the seeds of each species were thoroughly mixed before selections were made. The seeds to be used were then carefully selected from these lots for uniformity of size, shape, and general appearance. Only healthy appearing seeds were used. The seeds having a waxy or oily covering, like rape, were stirred in the solutions at first to prevent floating.

The influence of single-salt solutions in concentrations of 0.001 to 7.0 atmospheres upon the absorption was studied and the data are recorded in table 3. The amount of absorption from solutions by seeds of the different species varies considerably. The same is true for absorption in distilled water, but for seeds of the same species there is a marked degree of uniformity in the absorption from distilled water throughout all the series. The slight differences occurring may be attributed to the differences in temperature at which the experiments were carried out. The experiments were conducted during the period from June to November, and although the temperature in the laboratory did not change as did the temperature outside, nevertheless considerable variations in temperature were unavoidable. It was found that all the controls of like series which were carried out at approximately the same temperature showed no marked differences in the per cent of water absorbed, the same being true with respect to absorption from solutions.

The average per cent of absorption in distilled water during the 15-hour period of immersion for the different seeds was 43.4 per cent for wheat, 30.4 per cent for corn, 80.5 per cent for watermelon, 47.7 per cent for buckwheat, 90.8 per cent for Canada field peas, 139.7 per cent for white lupine, 118.7 per cent for soybeans, 56.9 per cent for rape, and 144.5 per cent for alfalfa. It is thus apparent that the nature of the seeds (structure, chemical and physical properties, etc.) exercises a very strong influence in determining the rate of absorption. Seeds of the leguminous type show higher absorption rates than do the seeds of other types here employed. The highest absorption rates are indicated for alfalfa, and the lowest for corn. It is to be expected also that the size of the seeds of the different species influences their rates of absorption, since small seeds such as alfalfa present a very much greater total absorbing surface per gram of seed than do the seeds of the larger types such as soybeans or white lupine. It is clear, however, from the data of table 3, that the size of the seed is by no means the predominating influence in determining the rates of absorption.

Inspection of table 3 brings out the fact that the general effect of increasing the osmotic concentration of the solutions is to decrease the absorption. This

TABLE 3

Absorption from single-salt solutions of varying osmotic concentration values relative to the absorption per gram of dry seed in distilled water taken as unity; all seeds immersed for a period of 15 hours

CONCENTRATION	WHEAT	CORN	WATER-MELON	BUCK-WHEAT	CANADA FIELD PEAS	WHITE LUPINE	SOYBEANS	RAPE	ALFALFA
Series 1, K ₂ CO ₃									
atm.									
7.000	0.93	1.00	0.96	0.98	0.93	1.02	0.95	1.00	1.38
6.000	0.94	1.00	0.99	0.88	0.96	1.03	0.93	0.99	1.20
5.000	0.96	1.01	1.02	0.94	0.97	1.04	0.93	0.98	1.06
4.000	0.99	1.03	1.04	1.00	0.99	1.01	0.96	1.05	1.09
3.000	0.99	1.02	1.03	1.00	0.97	1.01	0.97	1.05	1.13
2.000	0.98	1.07	1.08	1.02	0.99	1.00	0.97	1.05	0.98
1.000	0.98	1.06	1.04	0.99	0.99	0.98	0.98	1.05	0.92
0.500	1.01	1.10	1.01	0.98	0.99	1.00	0.97	0.99	1.04
0.100	1.02	1.00	0.96	1.02	0.98	0.98	0.98	0.96	1.04
0.010	1.00	1.01	0.98	1.05	0.99	0.99	0.98	0.94	1.06
0.001	1.00	0.99	0.97	1.06	1.00	1.01	0.99	0.99	1.03
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	*(0.443)	(0.302)	(0.786)	(0.464)	(0.890)	(1.451)	(1.237)	(0.591)	(1.442)
Series 2, Ca(NO ₃) ₂									
7.000	0.94	0.88	0.88	0.94	0.92	0.84	0.87	0.89	0.73
6.000	0.94	0.88	0.90	0.93	0.94	0.85	0.89	0.95	0.72
5.000	0.91	0.88	0.88	0.91	0.96	0.86	0.89	0.95	0.71
4.000	0.93	0.90	0.92	0.94	0.94	0.89	0.90	0.97	0.75
3.000	1.00	0.90	0.91	1.02	0.93	0.91	0.89	0.97	0.76
2.000	1.00	0.91	0.90	0.96	0.96	0.92	0.93	0.97	0.75
1.000	1.02	0.89	0.89	0.98	0.96	0.93	0.94	0.97	0.80
0.500	1.03	0.97	0.92	1.02	1.00	0.92	0.94	0.97	0.81
0.100	1.02	0.99	0.96	1.01	1.01	0.99	0.99	0.96	0.77
0.010	1.02	0.98	0.98	1.11	1.00	1.00	1.00	0.96	0.88
0.001	0.97	0.99	1.03	1.06	1.00	1.00	1.00	0.98	0.91
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.455)	(0.356)	(0.798)	(0.478)	(0.977)	(1.469)	(1.242)	(0.550)	(1.560)
Series 3, MgSO ₄									
7.000	0.79	0.78	0.83	0.89	0.95	0.81	0.87	0.91	0.74
6.000	0.85	0.82	0.85	0.93	0.95	0.85	0.87	0.95	0.76
5.000	0.85	0.87	0.86	0.95	0.95	0.89	0.90	0.95	0.84
4.000	0.85	0.86	0.90	0.94	0.97	0.91	0.94	0.97	0.81
3.000	0.88	0.88	0.90	0.98	0.97	0.92	0.93	0.97	0.91
2.000	0.88	0.88	0.90	0.97	0.98	0.97	0.94	0.99	0.87
1.000	0.92	0.91	0.92	1.05	1.01	0.98	0.94	1.01	0.90
0.500	0.92	0.95	0.93	1.00	1.03	0.96	0.95	1.00	0.91
0.100	0.94	0.95	0.94	1.09	1.02	0.99	0.97	1.06	0.98
0.010	0.97	0.98	1.02	1.08	1.01	1.02	0.99	1.02	1.00
0.001	0.96	0.99	0.97	1.05	1.02	1.01	0.99	1.03	1.05
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.477)	(0.445)	(0.905)	(0.453)	(0.867)	(1.340)	(1.215)	(0.580)	(1.405)

TABLE 3—*Continued*

CONCENTRATION	WHEAT	CORN	WATER-MELON	BUCK-WHEAT	CANADA FIELD PEAS	WHITE LUPINE	SOYBEANS	RAPE	ALFALFA
Series 4, NaCl									
7.000	0.95	0.88	0.80	0.81	0.92	0.87	0.91	0.95	0.82
6.000	0.96	0.88	0.84	0.83	0.94	0.89	0.93	0.99	0.85
5.000	0.99	0.88	0.90	0.92	0.94	0.92	0.93	0.92	0.85
4.000	1.01	0.91	0.90	0.86	0.94	0.93	0.93	0.93	0.92
3.000	0.99	0.95	0.90	0.89	0.96	0.93	0.95	0.93	0.84
2.000	1.01	0.98	0.88	0.93	0.97	0.96	0.96	0.93	0.88
1.000	1.04	0.95	0.98	1.02	1.00	0.99	0.97	1.02	0.88
0.500	1.07	1.00	1.01	0.96	0.98	0.98	1.01	0.94	0.88
0.100	1.07	0.99	0.98	0.94	0.98	1.00	1.01	0.95	0.99
0.010	1.05	1.02	1.00	1.01	0.96	1.01	1.01	0.95	0.97
0.001	1.05	0.98	0.94	1.01	0.98	1.02	1.01	0.95	1.01
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.408)	(0.284)	(0.813)	(0.492)	(0.932)	(1.341)	(1.181)	(0.612)	(1.460)
Series 5, KCl									
7.000	0.97	0.88	0.75	0.90	0.93	0.89	0.85	0.94	0.81
6.000	0.96	0.90	0.78	0.89	0.94	0.90	0.88	0.97	0.82
5.000	0.90	0.95	0.80	0.89	0.96	0.92	0.92	0.96	0.82
4.000	0.95	0.95	0.80	0.90	0.96	0.94	0.93	0.97	0.82
3.000	0.98	0.94	0.87	0.91	0.96	0.95	0.96	1.00	0.86
2.000	0.97	0.96	0.86	0.96	1.01	0.96	0.95	1.02	0.88
1.000	0.98	0.95	0.89	1.01	0.98	1.00	1.06	1.01	0.88
0.500	1.00	0.97	0.94	1.05	1.00	0.99	0.99	1.06	0.91
0.100	0.99	1.03	0.94	1.05	0.99	1.02	1.01	1.00	1.00
0.010	0.97	1.01	1.06	1.00	0.98	0.99	1.00	1.03	0.98
0.001	0.99	1.00	1.05	1.05	0.98	1.00	0.99	1.00	1.00
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.443)	(0.277)	(0.855)	(0.479)	(0.896)	(1.390)	(1.108)	(0.536)	(1.470)
Series 6, NaNO ₃									
7.000	0.95	0.91	0.89	0.86	0.97	0.89	0.90	1.00	0.84
6.000	0.95	0.93	0.90	0.85	0.99	0.92	0.89	1.00	0.88
5.000	0.92	0.93	0.97	0.87	1.00	0.91	0.93	0.96	0.89
4.000	0.97	0.93	0.97	0.91	0.99	0.93	0.96	1.02	0.91
3.000	0.96	0.97	0.96	0.91	1.00	0.93	0.96	1.06	0.88
2.000	0.95	0.97	1.01	0.92	1.01	0.93	0.96	1.13	0.95
1.000	0.97	0.97	1.02	0.93	1.02	0.95	0.97	1.02	0.90
0.500	0.97	0.96	1.01	0.97	1.04	0.97	1.00	1.06	0.90
0.100	0.97	0.99	0.96	0.96	1.04	0.96	1.01	1.07	0.93
0.010	0.96	0.99	1.00	0.96	1.03	0.96	1.02	1.04	0.94
0.001	0.97	0.99	1.01	0.96	1.03	0.96		1.03	0.96
0.000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(0.446)	(0.299)	(0.771)	(0.498)	(0.883)	(1.393)	(1.140)	(0.545)	(1.441)

* The actual amount of absorption per gram of dry seed in distilled water is given in parentheses in grams. The corresponding amount of absorption from any solution may be calculated by multiplying the relative weight by the actual weight absorbed in distilled water.

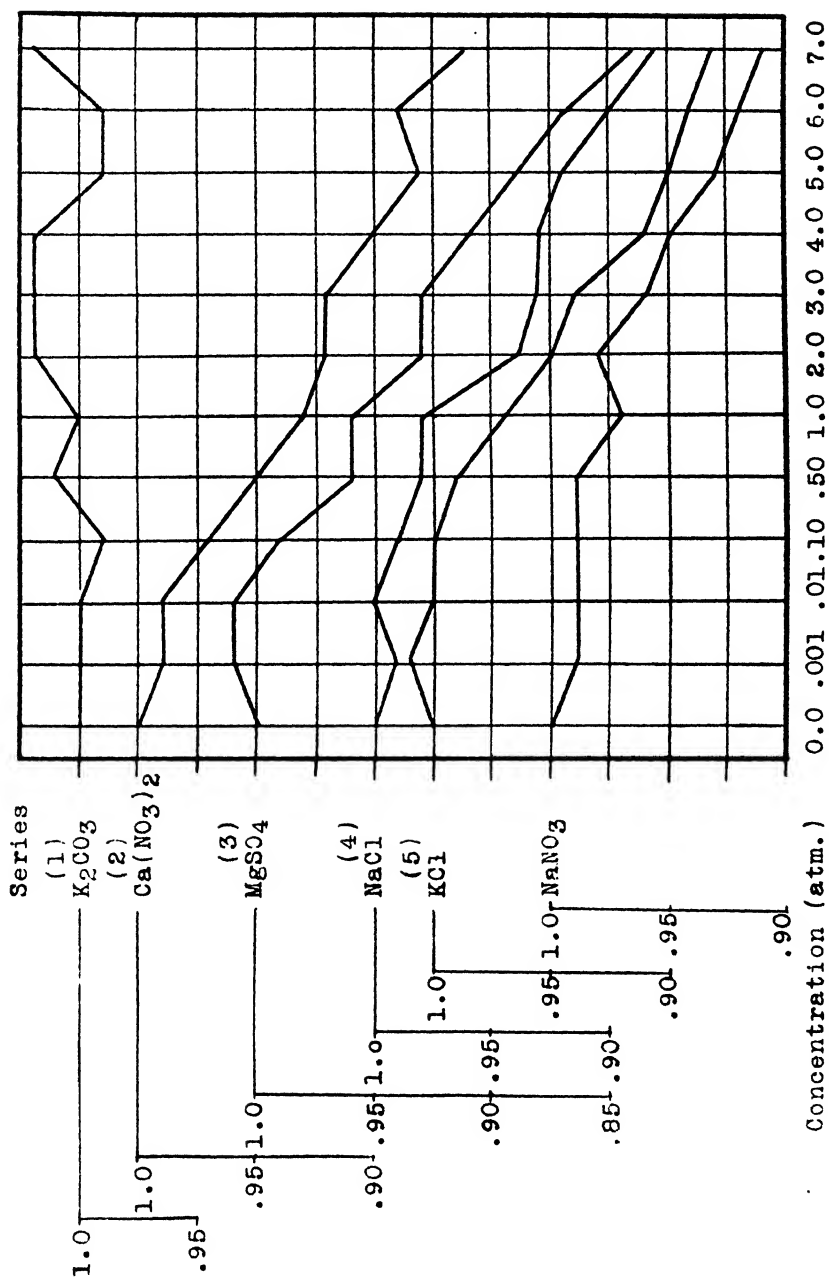
is true of all the different species of seeds in the solutions of all the salts used except K_2CO_3 . The decrease in absorption with an increase in the osmotic concentrations of the solutions is, of course, more uniform for some species of seeds than for others, and for any one species it is more uniform in some of the solutions than in others. It is to be noted, however, that low concentrations of some of the salt solutions appear to have a stimulating effect upon the absorption of some of the species, but not at all upon others. Thus, in the series with $MgSO_4$, there is indication that absorption by seeds of water-melon, buckwheat, Canada field peas, white lupine, rape, and alfalfa is stimulated by low concentrations of this salt, but absorption by wheat, corn, and soybeans is not thus affected. This stimulating effect upon the absorption by some of the seeds is fairly general throughout all of the series.

TABLE 4

Relative absorption data, being the average for the seeds of all species separately in single-salt solutions of corresponding concentrations of each salt; condensed summary of table 3

CONCENTRATION	K_2CO_3	$Ca(NO_3)_2$	$MgSO_4$	$NaCl$	KCl	$NaNO_3$
<i>atm.</i>						
7	1.02	0.86	0.84	0.88	0.88	0.91
6	0.99	0.89	0.87	0.90	0.89	0.92
5	0.99	0.88	0.89	0.92	0.90	0.93
4	1.02	0.90	0.91	0.93	0.91	0.95
3	1.02	0.92	0.93	0.93	0.94	0.96
2	1.02	0.92	0.93	0.94	0.95	0.89
1	1.00	0.93	0.96	0.98	0.97	0.97
0.5	1.01	0.95	0.96	0.98	0.99	0.99
0.1	0.99	0.97	0.99	0.99	1.00	0.99
0.01	1.00	0.99	1.01	1.00	1.00	0.99
0.001	1.00	0.99	1.01	0.99	1.01	0.99
0.00	1.00	1.00	1.00	1.00	1.00	1.00

Absorption in the K_2CO_3 series was very irregular. The solutions of this salt, being strongly alkaline in reaction, had the effect of softening not only the seed-coats but also the seeds themselves, the softening effect increasing proportionally with the concentration of the solutions. This chemical attack upon the seed-coats and seeds had a marked effect upon the rate of absorption, as is clearly indicated in the case of alfalfa. Absorption by this seed rapidly increased with an increase in concentration of the solution, the greatest rate of absorption occurring with the highest concentration. Other seeds, such as the white lupine and rape, were somewhat similarly affected, while wheat and Canada field peas appeared to be but very slightly influenced. The solutions of K_2CO_3 further exhibited a strong tendency to extract coloring matter from the seed-coats, a property which appeared to be entirely lacking in the solutions of all the other salts used. The seeds having colored pigments in their coats thus imparted to the solutions of this salt characteristic colorations, the degree of color depending upon the concentration of the solutions.



The general effect of salt concentration upon absorption is still further brought out by the data of table 4, which shows the average relative absorption quantities of the seeds of all species separately in corresponding concentrations of each single salt employed. These data, being a condensed summary of table 3, are represented graphically in figure 1. The average relative absorption amounts are here plotted as ordinates against osmotic concentration values in atmospheres as abscissas.

It will be observed that the graphs of all the series except that of series 1 (K_2CO_3) agree in showing a fairly uniform decrease in absorption quantities with an increase in the osmotic concentration values of the solutions. The stimulating effect of low osmotic concentrations upon absorption as brought out by the detailed data in table 3, is not clearly shown in these graphs, although the graphs of series 3 and series 5 do indicate slightly increased relative absorption quantities for some of the lower concentrations.

The chemical action upon the seed-coats and seeds of the solutions used in series 1 (K_2CO_3) and its consequent effect upon absorption from these solutions, as pointed out in connection with the data of table 3, is very clearly shown by the graph representing this series. This graph does not show the general downward slope to the right as do the graphs of the other series, while the average relative absorption from five different concentrations of the salt used in this series, including the highest concentration, is actually greater than that from distilled water (0.0 concentration).

II. Influence of time upon the absorption by seeds from solutions varying in osmotic concentration values

The conclusion drawn by Atkins (1) that "the rate at which distilled water is taken up is no greater than that at which salt solutions are absorbed" may hold for solutions with low osmotic concentrations; but with the higher concentrations used in the present study the absorption rates were decreased to a marked degree, as is clearly shown by the results of the preceding experiments. In order to determine whether the rates of absorption by the seeds would tend to equalize in solutions of different concentrations with continued immersion, experiments were carried out in which the periods of soaking varied from 15 to 149 hours. For convenience, only the larger seeds, corn, watermelon and beans, were employed. Single-salt solutions of $Ca(NO_3)_2$, KCl, KNO_3 , KH_2PO_4 , and K_2CO_3 were used. Tests were also made with Shive's three-salt solution, number R_5C_2 . The osmotic concentration values of the solutions in each series varied from 0.5 to 7.0 atmospheres and each series included controls with distilled water.

The seeds were soaked for a period of 15 hours in the manner previously described. At the end of this period each lot of seeds was removed from the solution and placed between double layers of filter paper saturated with the solution in question, and the whole was then placed in a moist chamber. Care was taken to keep the layers of filter paper enclosing each lot of seeds con-

stantly saturated with the proper solution during the entire period of incubation. Weights were recorded and the absorption amounts per gram of dry substance were calculated as before. The results obtained are presented in table 5.

TABLE 5

Influence of length of time upon the relative absorption of seeds in single-salt solutions of varying osmotic concentration values; absorption from distilled water per gram of dry seed considered as 1.00

CONCENTRATION	KCl								
	Beans			Corn			Watermelon		
	29 hrs.	48 hrs.	72 hrs.	15 hrs.	68 hrs.		15 hrs.	71 hrs.	96 hrs.
atm.									
7.0	0.87	0.86	0.83	0.88	0.64		0.75	0.56	0.70
6.0	0.91	1.00	0.87	0.90	0.70		0.78	0.70	0.70
5.0	0.94	1.10	0.89	0.95	0.77		0.80	0.77	0.71
4.0	0.97	0.97	0.93	0.95	0.83		0.80	0.75	0.75
3.0	0.92	0.94	1.00	0.94	0.86		0.87	0.79	0.82
2.0	0.95	0.93	1.01	0.97	0.92		0.86	0.88	0.95
1.0	0.96	1.06	1.02	0.95	0.93		0.89	0.99	1.08
0.5	1.03	1.27	1.07	0.97	0.98		0.94	0.97	1.14
0.0	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00
	(1.140)	(1.217)	(1.432)	(0.277)	(0.506)		(0.855)	(0.909)	(1.022)
	Ca(NO ₃) ₂								
	30 hrs.	48 hrs.	51 hrs.	15 hrs.	70 hrs.		15 hrs.	120 hrs.	
7.0	0.89	0.77	0.74	0.94	0.45		0.88	0.58	
6.0	0.91	0.80	0.79	0.94	0.47		0.90	0.75	
5.0	0.92	0.84	0.78	0.91	0.54		0.88	0.77	
4.0	0.93	0.86	0.80	0.93	0.60		0.92	0.64	
3.0	0.95	0.88	1.01	1.00	0.66		0.91	0.67	
2.0	0.96	0.92	0.89	1.00	0.85		0.90	0.90	
1.0	0.99	1.04	1.02	1.02	1.02		0.89	0.79	
0.5	0.99		1.15	1.03	0.99		0.92	1.00	
0.0	1.00	1.00	1.00	1.00	1.00		1.00	1.00	
	(0.932)	(1.124)	(1.176)	(0.455)	(0.818)		(0.798)	(0.989)	
	KH ₂ PO ₄								
	43 hrs.	68 hrs.		47 hrs.	69 hrs.		15 hrs.	119 hrs.	149 hrs.
7.0	0.86	0.91		0.73	0.65		0.82	0.75	0.49
6.0	0.96	0.87		0.77	0.70		0.83	0.86	0.52
5.0	0.91	0.90		0.79	0.73		0.86	0.81	0.51
4.0	0.90	0.93			0.73		0.86	1.16	0.79
3.0	0.93	0.93		0.89	0.89		0.87	1.29	0.92
2.0	0.96	0.96		0.89	0.96		0.92	1.45	0.98
1.0	1.04	0.92		1.03	0.89		0.94	1.80	1.05
0.5	1.02	0.93		1.02	1.11		1.02	1.18	0.94
0.0	1.00	1.00		1.00	1.00		1.00	1.00	1.00
	(1.191)	(1.291)		(0.459)	(0.628)		(0.648)	(0.907)	(1.439)

TABLE 5—*Continued*

CONCENTRATION	KNO ₃								
	Beans			Corn			Watermelon		
	42 hrs.	72 hrs.		15 hrs.	49 hrs.	52 hrs.			
<i>atm</i>									
7.0	0.86	0.80		0.90	0.83	0.69			
6.0	0.86	0.83		0.92	0.76	0.77			
5.0	0.94	0.81		0.92	0.85	0.83			
4.0	0.90	0.91		0.92	0.80	0.89			
3.0	0.94	0.92		0.96	0.85	0.95			
2.0	0.98	1.00		0.96	0.91	1.00			
1.0	1.02	1.01		0.96	0.94	1.03			
0.5	1.01	1.00		0.96	1.02	1.01			
0.0	1.00	1.00		1.00	1.00	1.00			
	(0.994)	(1.228)		(0.342)	(0.468)	(0.602)			
	MgSO ₄								
	15 hrs.	48 hrs.		15 hrs.	50 hrs.	68 hrs.			
7.0	0.86	0.89		0.78	0.67	0.47			
6.0	0.86	0.90		0.82		0.47			
5.0	0.89	0.90		0.87	0.69	0.50			
4.0	0.94	0.96		0.86	0.70	0.53			
3.0	0.92	0.99		0.88	0.75	0.61			
2.0	0.93	1.00		0.88	0.60	0.63			
1.0	0.93	1.00		0.91	0.79	0.79			
0.5	0.98	1.01		0.96	0.87	0.88			
0.0	1.00	1.00		1.00	1.00	1.00			
	(0.724)	(0.955)		(0.445)	(0.503)	(0.806)			
	K ₂ CO ₃								
	50 hrs.	68 hrs.		15 hrs.	55 hrs.	72 hrs.			
7.0	0.86	0.93		1.00	0.70	0.52			
6.0	0.92	0.90		1.00	0.70	0.54			
5.0	0.93	0.91		1.01	0.74	0.59			
4.0	0.99	0.84		1.03		0.61			
3.0	0.97	0.92		1.02	0.86	0.69			
2.0	0.93	0.93		1.07	0.91	0.74			
1.0	0.99	1.01		1.06	0.98	0.89			
0.5	1.00	0.96		1.10	0.93	0.83			
0.0	1.00	1.00		1.00	1.00	1.00			
	(1.095)	(1.360)		(0.302)	(0.507)	(0.679)			

TABLE 5—Continued

CONCENTRATION	Shive's three-salt solution R_3C_2							
	Beans			Corn			Watermelon	
	15 hrs.	48 hrs.		47 hrs.	67 hrs.		24 hrs.	113 hrs.
<i>atm.</i>								
7.0	0.92	0.87		0.84	0.73		0.87	0.65
6.0	0.94	0.93		0.85	0.74		0.91	0.61
5.0	0.97	0.94		0.88	0.80		0.93	0.72
4.0	0.97	0.94		0.86	0.82		0.93	0.80
3.0	1.01	0.91		0.88	0.84		0.97	0.70
2.0	1.00	0.92		0.91	0.96		0.98	0.80
1.0	1.02	0.94		0.94	0.91		0.98	0.86
0.5	1.00	0.99		1.03	1.22		0.96	0.77
0.0	1.00	1.00		1.00	1.00		1.00	1.00
	(1.036)	(1.188)		(0.403)	(0.602)		(0.668)	(1.147)

It should be stated here that the seeds soaked for the longer periods of time made considerable growth, and this perhaps may have had some slight influence upon the results obtained. It is of interest to point out that by far the greatest amounts of growth were made from the seeds in the solutions of lower concentrations, as indicated by measurements of the lengths of roots and shoots produced. Germination and growth rates were increasingly retarded with an increase in the osmotic concentrations of the solutions. Special attention has been given to this phase of the problem, which will be treated in detail in another study.

The data of table 5 show the same general relations between absorption rates and the osmotic concentration values of the solutions used as do the data of table 3. With longer incubation periods, however, the relative absorption amounts show more pronounced differences in passing from the lower to the higher concentrations, than they do with the shorter periods. This is true, in general, throughout all the series, including the series in which mixed solutions (Shive's three-salt solution R_3C_2) were employed. This influence of the time element upon absorption rates is more clearly brought out by the data of table 6 and by the graphs of figure 2. The data of table 6, which is a summary of table 5, represent the average relative absorption amounts for the seeds of each species separately, for the short and for the long absorption periods in all the solutions of corresponding concentrations. The average time of the short and of the long absorption periods is given at the head of the respective columns in the table. Each pair of graphs in figure 2 represents the average relative absorption data for a single species, the continuous line and the dotted line indicating the data for the short and for the long absorption periods, respectively. The absorption amounts as ordinates are here plotted against the osmotic concentration values as abscissas.

It will be observed that the graphs of each pair show considerable divergence, the lower one of each pair representing the average relative absorption amounts for the long-time periods. This divergence of the graphs is clear evidence that the rates of absorption by the seeds from solutions of different osmotic concentrations do not tend to equalize when the seeds are exposed to the solutions for longer periods of time. On the contrary, the graphs bring out the fact that the retarding influence of the solutions of higher concentrations upon the rates of absorption is more pronounced for the longer absorption periods than it is for the shorter.

From the preceding experiments it is clear that the general effect of increasing the osmotic concentration of the solutions is to retard the rate of imbibition by seeds in contact with them irrespective of the kind or nature of the salts

TABLE 6

Relative absorption data, being the average for seeds of each species in all the solutions of corresponding concentration but for time periods of different average lengths, condensed summary of table 5

CONCENTRATION	BEANS		CORN		WATERMELON	
	Average of short-time periods, 32.0 hrs.	Average of long-time periods, 58.7 hrs.	Average of short-time periods, 24.1 hrs.	Average of long-time periods, 66.1 hrs.	Average of short-time periods, 17.2 hrs.	Average of long-time periods, 119.5 hrs.
<i>atm.</i>						
7.0	0.88	0.85	0.87	0.59	0.83	0.60
6.0	0.92	0.87	0.89	0.63	0.85	0.64
5.0	0.93	0.88	0.90	0.65	0.87	0.68
4.0	0.94	0.90	0.93	0.72	0.88	0.74
3.0	0.95	0.95	0.94	0.79	0.90	0.78
2.0	0.96	0.96	0.95	0.97	0.91	0.91
1.0	0.99	0.99	0.98	0.92	0.93	0.93
0.5	1.00	1.02	1.01	0.99	0.96	0.96
0.0	1.00	1.00	1.00	1.00	1.00	1.00

employed, provided they do not react in solution with the seed substance to modify in a chemical way the imbibing properties of the seed as do the solutions of potassium carbonate. Dachnowski (3) has pointed out that while seeds show a greater water content in solutions of acids and alkalis than they do in distilled water, an optimal point is reached beyond which a further concentration of the acid or alkali is not followed by a greater water content but by a lesser one. The author states that no conception of colloidal swelling alone can be brought into harmony with the maximal values of water retained nor with the chemical changes which actually take place through which seeds are progressively altered. It thus appears that the retarding influence upon the rate of imbibition by the salts here employed is accomplished through the osmotic resistance offered to water entrance into the seeds. This is in entire accord with the conclusion of Slosson (5) and Buffum (2).

The average absorption rates from the salt solutions show an approximately linear relation to the osmotic concentrations of the solutions, decreasing with an increase in the concentration except in dilute solutions of some of the salts which appear to have a stimulating effect upon the rate of imbibition of the

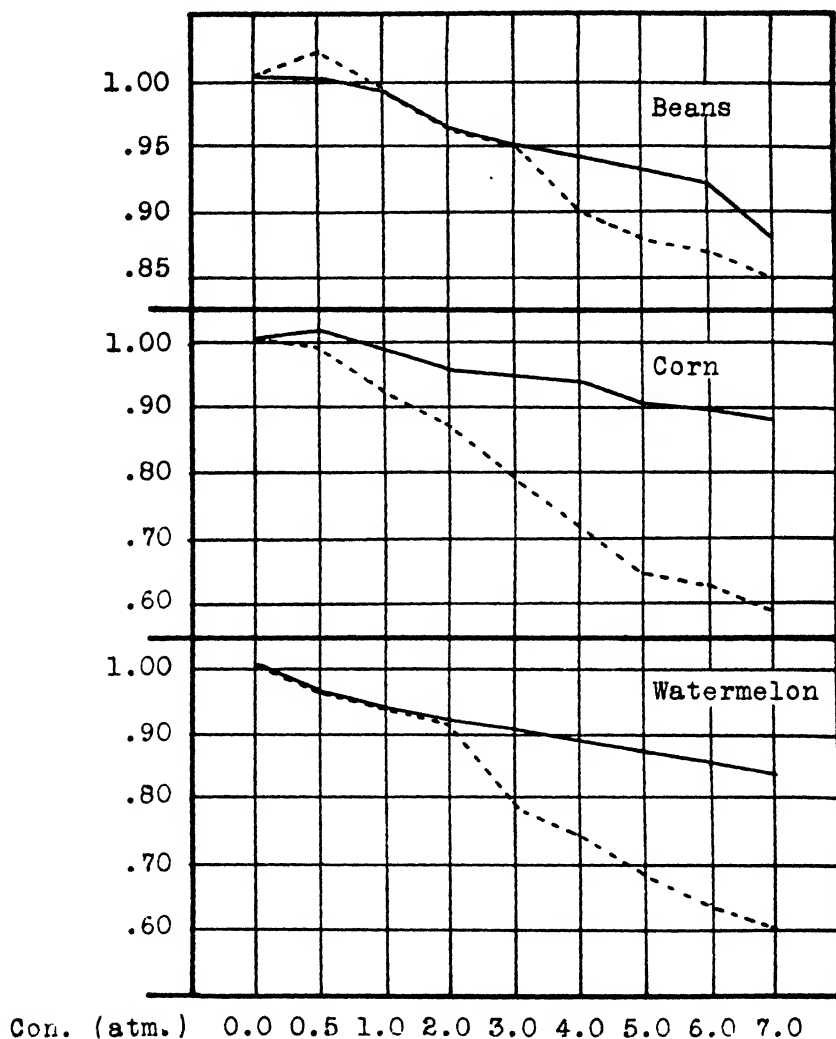


FIG. 2. GRAPHS SHOWING THE INFLUENCE OF TIME UPON RELATIVE ABSORPTION QUANTITIES IN SOLUTIONS OF VARYING OSMOTIC CONCENTRATIONS

Upper graph of each pair represents average absorption for short-time periods, lower graph for long-time periods.

seeds of certain species but not upon the rate of others. The physiological significance of these accelerated absorption rates in low concentrations of the salts employed has not been determined nor is it entirely clear from the data at hand whether any such significance can be attached to them.

SUMMARY

A study was made of the influence of salt solutions of varying osmotic concentrations upon the absorption by seeds of different species in contact with them. Single-salt solutions of $\text{Ca}(\text{NO}_3)_2$, K_2CO_3 , MgSO_4 , KH_2PO_4 , NaCl , KCl , and NaNO_3 were employed. A complete nutrient solution for plants (Shive's three-salt solution no. R_3C_2) also was used. The osmotic concentration values of the solutions of each salt were made to vary from 0.001 atmosphere to 7.0 atmospheres. Seeds of wheat, corn, watermelon, buckwheat, Canada field peas, white lupine, soybeans, rape, and alfalfa were immersed in the solutions for definite periods of time and the amounts of absorption from the solutions and from distilled water were determined. The influence of time upon the absorption from the solutions and from distilled water also was studied.

The results of the experiments may be summarized briefly as follows:

1. There is a marked difference in the absorbing power of seeds of different species. Seeds of the leguminous type show higher rates of absorption than do seeds of the other types used. The highest absorption rates are indicated for alfalfa, the lowest for corn.
2. The rates of absorption are progressively retarded with an increase in the osmotic concentration values of the solutions which do not react with the seed substance to modify in a chemical way the imbibing properties of the seeds.
3. Average absorption rates show an approximately linear relation to the osmotic concentration values of the solutions, decreasing with an increase in concentration except in dilute solutions.
4. Experimental evidence points to the conclusion that retardation of the absorption rates is accomplished through osmotic resistance offered to water entrance into the seed.
5. Low osmotic concentrations appear to have a stimulating influence upon the absorption rates of the seeds of some species but not upon the rates of others. The physiological significance of these accelerated absorption rates in solutions of low osmotic concentration values has not been determined.
6. Increasing the length of the time period during which the seeds are in contact with the solutions has the effect of increasing the differences between the quantities absorbed from solutions of low and from those of high osmotic concentration values.

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A COMPARISON OF THE TECHNIC RECOMMENDED BY VARIOUS AUTHORS FOR QUANTITATIVE BACTERIOLOGICAL ANALYSIS OF SOIL

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A quantitative bacteriological analysis of soil for total numbers of microorganisms has but a comparatively small significance as compared with that for the estimation of numbers in one or more physiological groups. Remy (25) and Hiltner and Störmer (18) recognized this as early as 1902-1903. The principles underlying the technic used for the enumeration of total numbers of microorganisms, however, lend themselves as well to the estimation of numbers in one or more physiological groups, the only difference being in the media employed (18, 28).

Certain details of the technic recommended by various authors for analyzing soil bacteriologically are widely divergent, so much so in fact that the data obtained from one soil analyzed simultaneously by the various methods would be wholly incomparable. Up to the present time each investigator has been a law unto himself in so far as the technic used in quantitative bacteriological soil analysis is concerned. For example, King and Doryland (20) used the volumetric method, i.e., adding 19 cc. of diluting liquid to 1 cc. of soil, stating that "This method is undoubtedly inaccurate, as it is difficult to measure such substances as soil in terms of cubic centimeters. However, for the purpose of securing comparative results, as in the present work, either method may be used. . . . The final results would appear to be correct so long as one or the other method of collecting samples is followed with uniformity throughout the work." (By the method of "collecting" samples is meant the volumetric or gravimetric methods.)

Kellerman and Allen (19) state "Though it is recognized that the methods suggested by different investigators are not adequate for accurate quantitative investigations of bacterial functions and conditions in various soils, the methods which at this time have been found most convenient and suitable for the investigations under discussion are briefly reviewed."

Bacteriologists who have experimented with soils to a considerable extent appreciate the fact that even when any one method or technic is employed, quantitative results are not infrequently very erratic. Chester (4) realized that unless one method is used for quantitative bacteriological soil analysis, comparative results could not be obtained. To quote, "In analyses of other soils, these same conditions should be duplicated, otherwise reliable comparative results cannot be expected."

A comparison has been made of the quantitative soil bacteriological methods recommended by the authors of soil bacteriology and other laboratory manuals, and also of comparatively recent articles dealing with or using quantitative methods for bacteriological soil analysis. This brings to light the information that, at the present time, no one method is used. The several points about which the various authors differ are as follows:

(a) The amount of soil used; (b) the proportion of the soil to the diluting liquid in the first dilution; (c) the size of the second dilution; (d) the method of making the first dilution; (e) the technic for transferring dilutions containing visible amounts of soil; (f) the basis for the estimation of numbers (total or otherwise).

These points of difference will be taken up in order.

Amounts of soil varying from 0.25 gm. to 100 gm. are used in the first dilution.

TABLE 1
Variations in amounts of soil used in first dilutions

NUMBER OF RECOMMENDA- TIONS	FIRST DILUTION		AUTHORS
	Soil used	Approximate amount of diluting liquid	
	gm.	cc.	
1	0.25	50	Ellis (8)
1	0.5	100	Chester (4), Conn (6)
7	1.0	99	Gainey and Gibbs (12)
		100	Savage (26), Schneider (27)
		300	Kellerman and Allen (19)
		1,000	Frost (11), Hastings and Wright (15), Löhnis (21)
2	10.0	100	Eyre (9), Heinemann (16)
3	20.0	200	Fred (10), Giltner (13), Hills (17)
1	50.0	200	Noyes and Voigt (24)
1		500	Remy (25)
4	100.0	200	Brown (2), Burgess (3), Greaves and Carter (14), Whiting (31)
1	Not stated		Waksman (29)
1	1 cc.	19	King and Doryland (20)

The proportion of soil to diluting liquid employed in the first and second dilutions varies as shown in table 2.

No attention was paid by the author of this paper as to how further dilutions were made, as the first two are deemed the most important from the standpoint of the technic in making plates or other cultures.

With four exceptions, the first dilution, almost as if by common consent, is made by adding the weighed soil to the measured amount of water. Chester, Gainey and Gibbs, Heinemann, and King and Doryland are the only ones who consider the weight or volume of the soil in making the first dilution. Gainey and Gibbs prepare the first dilution by adding 1 or 2 gm. of soil to 99 or 98

cc. of dilution liquid as desired. King and Doryland add 1 cc. of soil to 19 cc. of diluting liquid. Chester, and Heinemann, on the other hand, are the only ones who employ the technic recognized in pharmaceutical practice. They prepare the first dilution by triturating the soil (0.5 and 10 gm., respectively)

TABLE 2
Variations in proportion of soil to diluting liquid in first and second dilutions

NUMBER OF RECOMMENDATIONS	FIRST DILUTION	SECOND DILUTION	MADE BY PLACING	AUTHORS
3	1:2	1:100 1:200 1:2,000	2 cc. + 98 cc. 1 cc. + 99 cc. Not stated	Whiting Burgess, Greaves and Carter Brown
1	1:4	1:40	10 cc. + 90 cc.	Noyes and Voigt
7	1:10	1:100 1:1,000 1:2,000 Not stated	1 cc. + 9 cc. 10 cc. + 90 cc. 1 cc. + 99 cc. 1 cc. + 199 cc. Not stated	Eyre Fred, Giltner Heinemann Waksman Remy
1	1:20	1:2,000	Plates loopful using platinum loop of 0.001 cc. capacity	King and Doryland
1	1:50	*		Gainey and Gibbs
3	1:100	* 1:1,000 or less 1:10,000	1 cc. or less + 9 cc. or more 1 cc. + 99 cc.	Gainey and Gibbs Schneider Savage
3	1:200	Not stated 1:800 1:20,000	Not stated Plates 0.25 cc. 1 cc. + q. s. = 100 cc.	Conn Ellis Chester
1	1:300	None	Plates 1 cc. of first dilution	Kellerman and Allen
3	1:1,000	1:2,000 1:10,000	Plates 0.5 cc. 1 cc. + 9 cc.	Frost Hastings and Wright, Löhnis

* Dilutions made in the "ordinary way."

in a mortar with a small amount of the diluting liquid, then add a sufficient quantity of the diluting liquid to make a total of 100 cc. With the exception of Schneider who triturates 1 gm. with 100 cc. of diluting liquid, all other authors reviewed here mix the soil with the diluting liquid merely by shaking for a specified time or number of times.

The size of the second dilution is important also. As with the first, an attempt should be made to obtain as representative a sample as possible for transfer into the second dilution flask. This is not possible when much less than one-tenth of the soil in the first dilution is transferred to the second dilution flask. By consulting tables 1 and 2 it will be seen that Fred and Giltner are the only ones who recommend the ideal (according to the writer's idea) first and second dilutions (1:10 and 1:100, respectively). In fact, these dilutions should be credited to Fred alone, as the writer used Fred's dilutions, considering them as ideal, in writing up this experiment for Giltner's Manual. Schneider's method which recommends 1:100 as the first dilution and 1:1000 as the second, is next in desirability. Noyes and Voigt state that the second and succeeding dilutions should differ from one another by 10. They also show by experiment that considerable error is eliminated when 10 cc. of a dilution is added to 90 cc. of the diluting liquid for making the succeeding dilution rather than by adding 1 cc. to 9 cc., proving that the results of working the two ways are not the same. The writer agrees almost wholly with Noyes and Voigt, with the exception that when a dilution becomes high enough to have eliminated all visible soil particles there seems to be no reason why succeeding dilutions should not be made by transferring 1-cc. portions to 99 cc. of diluting liquid. This would not prevent making plates or other cultures from dilutions differing by 10.

The number of bacteria, in practically every case, is estimated "per gram of soil." Probably moist soil is meant in many instances although King and Doryland state that "it is the general custom of bacteriologists to compare the number of bacteria per gram of dry soil." The last mentioned authors express the number of bacteria in soil both as "per cubic centimeter" and "per gram" for purposes of comparison.

Twelve authors state their results definitely on the basis of dry soil (Brown, Chester, Conn, Cook (8), Eyre, Gainey and Gibbs, Heinemann, Hills, King and Doryland, Noyes and Voigt, Remy, and Schneider). Neither Conn, Hills, Schneider nor Noyes and Voigt in the publications here reviewed states how the soil is dried. Eyre dries 10 gm. of soil over a water bath to a constant weight; Gainey and Gibbs dry the soil at 110°C. for 2 hours; Heinemann dries the soil in the hot-air oven 1 hour at 100°C.; and Chester dries the soil for 3 hours at 100°C. On the other hand, Brown, King and Doryland, Cook, and Remy merely air-dry the soil.

This brings to consideration the value of the points in the technic of quantitative bacteriological soil analysis which differ so widely with the different authors.

First, what amount of soil should be employed in making the first dilution? It is well known among soil bacteriologists that in soil, probably more than in any other solid or semi-solid natural medium such as cheese, butter, finely chopped meat, curdled milk, and the like, a localization of physiological groups of bacteria and other microorganisms is a common occurrence. These

localizations do not depend entirely on the fact that the soil is more or less of a solid medium, as do similar localizations or, as it may be termed, colony formations, occurring in more homogeneous media. To the contrary they may depend on the composition of the soil particles with which they are in immediate contact. For example, the *Azotobacter* species is known to colonize around particles of calcium carbonate. It seems, then, that whatever method is employed in mixing the soil sample or however thoroughly it is mixed previous to making the first dilution, a sufficiently large amount should be taken to include colonizations as representative of all desired physiological groups as possible. For this reason samples as small as 1 gm. do not seem to be desirable. Löhnis recognized the importance of this point (23) in obtaining information concerning the transformations in the soil induced by micro-organisms. He could not secure satisfactory agreement in duplicates where only 0.5 to 2 gm. of soil were used for inoculation (28). He is inconsistent apparently, however, in recommending the use of 1-gm. samples in the determination of bacterial numbers in soil (21). Noyes and Voigt found in making moisture tests of soils for bacteriological analysis that "it took 10-gm. aliquots to have the duplicates check regularly to 0.1 per cent" and are forced to conclude that "it would take larger aliquots to get good bacteriological results than it would for good moisture results". They recommend 50 gm. of fresh soil as a standard. The type of soil might be used to determine within certain limits the amount taken. In the case of a soil like peat for instance, 100 gm. or more should be taken, depending upon the lack of homogeneity, while with a fine sand a 10-gm. sample might prove representative.

It might be well to obtain more or less of a uniformity in the size of the first dilution for all soils. As noted previously the first dilutions vary with different authors from 1:2 to 1:1000. A dilution of 1:2 may be satisfactory for soils like sand, sandy loam, and the like but for soils having high water-holding capacity, this low dilution would be highly undesirable. The lowest dilution which seems desirable for these latter soils is 1:10, which also gives a satisfactory dilution for the sandy soils, etc. An initial dilution of 1:100 also may prove satisfactory if a sufficiently large amount of soil is taken.

It is previously noted that the various authors have differing ideas of how to go about making a quantitative dilution of soil. Practically all of them take the amount of diluting liquid instead of the soil as a basis in determining the first dilution. Whichever method is correct is apparently a subject of controversy, but should it not be decided one way or the other?

In the lack of homogeneity, soil resembles certain other substances of which quantitative bacteriological analysis is made; for example, hamburger steak, shellfish, cheese and butter. Eyre (9) and Savage (26) obtain the first dilution (1:10) for quantitative bacteriological analysis of oysters, mussels and cockles by placing the comminuted shellfish with their liquor into a 1000-cc. graduate and making up the volume to 1000 cc.

Löhnis (21) dilutes cheese by making up 1 gm. of cheese (ground in a mortar with sand and 10 cc. of water) to 1000 cc.

Butter (13, 21) is diluted by placing 1 gm. into 99 cc. of warm water.

Weinzirl and Newton (30) grind a known weight of meat in a mortar with sand, as is done with cheese, and bring the volume up to the desired dilution.

These methods have been accepted as standard by practically all of the bacteriologists who have occasion to employ them for these or like substances. It seems, then, that soil which is not so markedly different from these other substances should be subjected to the same principle in making the initial dilution, that is, adding sufficient of the diluting liquid to make up the known weight of soil to the desired dilution.

The advisability of grinding the sample previous to making the dilution would appear to depend largely on the type of soil. It would not seem necessary to subject sand, clay and the like to the grinding process, as merely placing it in the diluting liquid would serve to separate the particles sufficiently. Soils containing appreciable amounts of organic matter, however, should be so treated before diluting that the diluting liquid will reach all of the particles in as short a time as possible. This probably would best be accomplished by grinding with a little of the diluting liquid.

Although the size of the remaining dilutions made in quantitative bacteriological soil analysis is important, the ones actually used will depend on the object of the quantitative test.

Right here one point is worthy of mention. Certain authors (Fred, Whiting, and perhaps others) recommend that the first and later dilution of soil be allowed to settle after shaking, before making further dilutions or plating. Does not the same principle hold with solid material to be analyzed as with liquid material? Is it not well known that every effort is put forth by the bacteriologist in making quantitative dilutions of milk to get 0.1 cc. of milk in every cubic centimeter of a 1:10 dilution by shaking thoroughly the dilution flask so that he transfers 0.1 cc. of actual milk in every cubic centimeter of the 1:10 dilution placed in the plate; or does he assume that the vigorous shaking has successfully dislodged the greatest percentage of microbes from the solid portion of the milk which then might be left behind in the flask (if it were possible) as well as not? Curdled milk presents a problem to the bacteriological analyst similar to that of soil. Looking at it in this light, does it not seem very illogical to assume that soil can be washed free of all or even of most of its organisms by the short shaking process employed? Again, are not the facts well known concerning the influence of sedimentation on bacterial numbers, a well-founded argument against allowing the soil to settle in the dilution?

A few authors have recognized this point. Conn (5) advised "care being taken to keep the contents of the flask in motion when any of the suspension was withdrawn." To quote Noyes and Voigt, "get the soil and water in the first bottle * * * thoroughly in motion by shaking and while the mixture is still in motion fill the pipette." Hastings and Wright, Löhnis (21),

and Burgess also state more or less definitely the necessity for keeping the dilution in motion while in the act of transferring portions of it.

The method of estimating numbers of bacteria, either the total count or the enumeration of any one physiological group, is very important. It does not seem presumptuous to state that with soil which fluctuates so much in its moisture content, even when "air-dry," the estimation of numbers should be on the oven-dry basis. Furthermore, it seems logical that the method for oven-drying the soil should be that recommended by the Association of Official Agricultural Chemists (1) in their latest report, i.e., "Dry 2 gm. of the sample . . . at 100°C. to constant weight." As the bacteriologist generally does not care to subject his soil sample to the pulverizing and sifting process recommended for the chemist, a much larger sample should be used to be representative. The important point is the method of drying. The bacterial content per gram of one sample of undried soil sampled at various times would fluctuate within a wide range due to the fluctuation in the moisture content, and most probably the same thing would be true, only in a much lesser degree, of air-dry soil.

The consideration of these points relative to the quantitative bacteriological analysis of soil completes the purpose of this article. It is not the intent of the writer in this paper to discuss apparatus or methods used in taking soil samples, methods of mixing the sample before making the first dilution, nor the particular dilution liquid, dilutions, or media to use. Most of these latter points have been thoroughly discussed previously by one or more authors with helpful results.

It is certain that most investigators or teachers who have had occasion to deal with quantitative bacteriological soil analysis will appreciate to a considerable degree the fact that the points brought up in this paper are some of the most important of those met with, and that an effort toward a standardization of certain fundamental features of the technic would be a desirable step.

SUMMARY

The following points are suggested as worthy of attention should the standardization of quantitative bacteriological soil analytical methods be considered at any future time.

1. Not less than 10 gm. of soil should be used in making the first dilution.
2. The initial dilution should be not less than 1-10.
3. If the soil contains considerable organic matter, it should be triturated in a mortar with a little of the diluting liquid.
4. In all cases the weighed soil sample should be made up to the volume of the desired initial dilution by the addition of sufficient diluting liquid, e.g., for a 1-10 dilution, 10 gm. of soil should be placed in the graduated flask, cylinder, etc., and sufficient diluting liquid added to bring the volume up to 100 cc.

5. Care should be taken in making further dilutions or in plating to transfer an aliquot of the soil itself, as nearly as possible.

6. The second dilution should contain not less than one-tenth of the amount of soil in the first dilution. Noyes and Voigt's recommendation may well be followed in making succeeding dilutions, i.e., each higher bacterial dilution should be made by taking 10 cc. of the lower bacterial dilution and 90 cc. of the diluting liquid.

7. Numbers of microorganisms (total or otherwise) should be estimated on the basis of soil oven-dried at 100°C. to a constant weight.

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SOME SOIL FUMIGATION EXPERIMENTS WITH PARADICHLOROBENZENE FOR THE CONTROL OF THE PEACH-TREE BORER, *SANNINOIDEA EXITIOSA* SAY.¹

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Since 1917 the author has conducted numerous and varied experiments for the control of the peach-tree borer. During 1918 to 1920 several soil fumigants were tried out. In 1918 and 1919 sodium cyanide was used extensively. The results of the experiments with sodium cyanide may be found in another paper (2). Briefly stated, the results show that sodium cyanide will give fair results under certain conditions. Generally speaking, the margin of safety, that is the difference between the killing point of the larvae and the killing point of the tree, is not sufficient for general practice. Furthermore, sodium cyanide is extremely poisonous to man.

During 1919 and 1920 paradichlorobenzene was used as a soil fumigant for the peach-tree borer. This paper gives a summary of the more important results we have obtained with this product. In November 1919, Blakeslee (1) published the results of his experiments with several soil fumigants. His results with paradichlorobenzene were very encouraging and they have spurred us on to learn all we could about it. The writer has had occasion frequently to refer to Blakeslee's splendid bulletin and it has been decidedly interesting to compare our results with his. For the most part the results of similar experiments agree.

Paradichlorobenzene is a white crystalline substance occurring as a by-product in the manufacture of monochlorobenzene. Paradichlorobenzene is practically insoluble in water. It evaporates slowly at ordinary temperatures (70°F). The gas coming from the crystals is heavier than air. The fumes are non-poisonous to man under ordinary conditions but are toxic to insects when the latter are exposed to them for a considerable period of time. If the directions given in the following pages are followed, paradichlorobenzene will kill 90 per cent or more of the larvae of *Sanninoidea exitiosa*. In a number of experiments 98 to 100 per cent of the peach-tree borers have been killed.

In our soil fumigation experiments with paradichlorobenzene for the control of the peach-tree borer, we have determined or we are determining the following phases of the problem:

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1. The amount of paradichlorobenzene needed to kill all of the small and large borers in trees of varying ages.
2. The effect of varying amounts of paradichlorobenzene on trees of different ages and varieties.
3. The killing effect of short exposures of small and large amounts of paradichlorobenzene on borers in trees younger than 6 years of age, and the response of young trees to short treatments.
4. The best time of the year to make applications.
5. The essential details of the method of application.
6. The influence of soil texture, soil acidity and alkalinity, soil moisture, and soil temperature on the rate of evaporation, and the effectiveness of paradichlorobenzene as a soil fumigant.
7. The cost of applications.
8. Other miscellaneous experiments.

1. STRENGTH OF THE APPLICATION

Our experiments show that prolonged exposures (21 days or more) of $\frac{3}{4}$ and 1 ounce of finely divided paradichlorobenzene per tree, kill 90 per cent or more of the worms present in 6-year old trees provided the soil conditions are favorable. One-half ounce in the vast majority of our experiments gave as satisfactory results as $\frac{3}{4}$ and 1-ounce treatments. Applications of $\frac{1}{8}$ and $\frac{1}{4}$ ounce are insufficient. At their best these strengths did not kill over 50 per cent of the larvae, and the larvae that were killed with $\frac{1}{8}$ and $\frac{1}{4}$ ounces were small, less than $\frac{1}{2}$ inch in length. Large larvae ($\frac{3}{4}$ inch or longer) are more difficult to kill than small larvae. Many of the large larvae are stupefied by small doses or short exposures of the gas, but they are not killed.

2. INJURY TO TREES

Trees 6 years of age or older are not seriously injured by the fumes of paradichlorobenzene if the applications are made when the soil is warm. Slight injury to the outside bark may take place. This usually occurs as a dark brown color in the bark. So far as our experience goes this injury is superficial and of no importance. In a few instances 6-year-old trees or older, which were heavily infested and damaged by borers, died after they were treated with paradichlorobenzene. These trees probably would have died even though they had not received the treatment. In other words, some trees which are damaged and greatly weakened by borers may not be saved by paradichlorobenzene. It is a debatable point whether such trees are killed by the treatment or by the borers. At the present time we are not certain what will happen to 6-year-old trees, or older, if they are subjected to long exposures of paradichlorobenzene when the soil temperature is 55°F. or colder. In November, 1919, we treated 20 trees in two orchards with $\frac{1}{2}$ and 1 ounce applications of paradichlorobenzene. Today these trees appear to

be normal and healthy. In a few cases we have injured (not killed) trees younger than 5 and 6 years of age with prolonged exposures of $\frac{1}{2}$ and 1 ounce of paradichlorobenzene. This is particularly true with trees 3 years of age or younger. Injury appears in the cambium layer as small brown specks, each about the size of the head of a pin. When serious, these spots converge and the entire living layer turns brown, becomes dry and dies.

To date our experiments do not show any variation in the susceptibility of different varieties of peaches to paradichlorobenzene. Only extensive experiments and observations over a considerable period of time can determine this point.

In case prolonged exposures of paradichlorobenzene prove to be satisfactory for trees 6 years of age or older, then it will be necessary to find some method of controlling borers in young trees. It is possible that short exposures of paradichlorobenzene will kill the worms in young trees and will not produce sufficient injury to the tender bark to be of importance.

3. SHORT EXPOSURES

During the past season experiments were conducted in several young peach orchards with the express purpose of determining the killing effect of short exposures (2, 4, 7, and 10 days) of $\frac{1}{2}$ ounce (or greater amounts) of

TABLE 1

Results of 2, 4, 7 and 10-day treatments with 0.5 ounce of paradichlorobenzene on 4-year-old flowering peach trees, in Penn loam soil at Bound Brook, N. J.

Ten trees in each treatment

NUMBER	DAYS EXPOSED	DATE TREATED	DATE REMOVED	LARVAE PER TREE		PERCENTAGE DEAD
				Alive	Dead	
1	*2	October 2	October 4	4.0	1.7	29
2	*4	September 25	September 29	3.1	5.4	63
3	*7	September 15	September 22	0.8	3.3	80
4	*10	September 15	September 25	1.4	3.7	72
5	†2	October 14	October 16	3.4	1.5	30
6	†4	October 14	October 18	2.6	2.9	52
7	†7	October 14	October 21	1.7	4.8	71
8	†10	October 14	October 25	0.5	4.6	90

* Soil temperature 62° to 58° F., moisture 10 to 15 per cent.

† Soil temperature 58° to 56° F., moisture 15 to 20 per cent.

Check trees—larvae all alive.

paradichlorobenzene on borers in trees younger than 6 years of age. The response of the young trees to short exposures also was observed. Table 1 summarizes the results of two series of experiments with heavily infested 4-year-old flowering peach trees subjected to $\frac{1}{2}$ ounce for 2, 4, 7 and 10 days. The table also records the nature and condition of the soil. The 7-day treat-

ments killed 71 to 80 per cent in these experiments while the 10-day treatments killed 72 to 90 per cent. These and other experiments of a similar nature show an average kill of 80 per cent for 10-day exposures with $\frac{1}{2}$ ounce. The great majority of the larvae which survived the 10-day treatments were $\frac{3}{4}$ inch in length or longer. In other words, the small larvae were killed. The author is of the opinion that it is more important to kill the small larvae than the large larvae because a small borer is capable of doing more damage than a full grown (or nearly grown) worm.

Several owners of peach orchards in New Jersey treated their young trees, 3 to 6 years of age, with $\frac{1}{2}$ ounce of paradichlorobenzene in September, 1920. One grower on September 17 treated 125 3-year-old and 600 4-year-old heavily infested Hiley trees with $\frac{1}{2}$ ounce and then removed the crystals and dirt to ground level in 7 days from the 3-year-old trees and in 12 days from the 4-year-old trees. The sandy soil in which the trees were located was warm and somewhat dry during the treatment. Thirty of the trees were examined in October, and an average of 0.1 living worm per tree was seen in the 3-year-old trees and less than 2.0 living worms per tree in the 4-year-old trees. No serious injury was noted; only one 3-year-old tree showed decided injury to the cambium layer about a cavity where a larva had been feeding. Our experiments to date show no serious injury to trees 3 to 6 years of age, where $\frac{1}{2}$ ounce of paradichlorobenzene was used for 7 to 10 days. We have injured nursery stock with $\frac{1}{2}$ ounce with 10 days' exposures, but the seriousness of this injury cannot be determined until 1921. After one or more years' experience with short exposures with varying amounts of paradichlorobenzene more definite statements can be made concerning the effectiveness and safeness of short exposures on young trees.

4. TIME OF APPLICATION—EARLY AND LATE SUMMER TREATMENTS

A series of experiments on the time of application were conducted in five orchards throughout the state. The experiments reported in table 2 were conducted at Bridgeton and the results are representative of all. About one-half of the trees in each series have been wormed; the others will be examined in 1921. All of the trees treated with $\frac{1}{2}$ and 1 ounce on June 25 (exp. 2 and 3) showed no larvae (100 per cent reduction) on July 27, while other trees treated at the same time but examined on October 7 possessed 14 to 15 larvae per tree (exp. 4 to 6). Comparing this infestation with the check trees the reduction approximated 30 per cent. Other trees which received two treatments, June 25 and August 5 (exp. 7 and 8) and were examined on October 7, showed an average of 0.4 or less larva per tree, a reduction of 98 per cent or better. Trees treated September 10 (exp. 9) and examined October 7 showed 99 per cent reduction. This series of experiments and others of a similar nature show that trees receiving two summer treatments or one late summer treatment will be free of borers during the fall, winter, spring and

early summer months, while trees receiving only one early summer treatment in June (or earlier) may possess some or many borers all months of the year except for a period of 6 to 8 weeks immediately following the treatment.

To date we have seen no injury to trees receiving two applications during the summer. Under some conditions two applications may prove to be desirable; however, further experiments and observations are necessary to determine the effect of two applications in one season on different varieties and under varying soil conditions.

Early spring applications are undesirable because the soil temperature is too low for the immediate and effective killing of the borers. Furthermore, the effect on trees of early applications in cold soils is unknown.

TABLE 2

Early and late summer treatments with paradichlorobenzene on Elberta peach trees, 12 years old, in sand to sandy loam soil at Bridgeton, N. J.

NUMBER	NUMBER OF TREES	AMOUNT APPLIED	DATE TREATED	DATE WORMED	LARVAE PER TREE ALIVE	REDUCTION
		oz.				per cent
1	10		Check	July 27	1.8	
2	10	1.0	June 25	July 27	0	100.0
3	10	0.5	June 25	July 27	0	100.0
4	5		Check	October 7	22.4	
5	*8	1.0	June 25	October 7	15.8	29.5
6	*5	0.5	June 25	October 7	14.8	34.4
7	*10	1.0	June 25			
			August 5	October 7	0.2	99.1
8	*5	0.5	June 25			
			August 5	October 7	0.4	98.5
9	10	0.75	September 10	October 7	0.2	99.1

* Twenty-five eggs placed on each tree.

In case applications are made after September 10 our present knowledge indicates that it is advisable to pull the dirt away from the trees to ground level where the crystals were placed in order that the gas may get away from the tree before the ground becomes cold and freezes. The dirt may be removed any time after the material has been on two or three weeks provided the soil has not been continuously wet and the temperature has been 55 to 60°F. or higher. In seasons when the weather is warm and dry during September and October most of the paradichlorobenzene will evaporate from the soil even though it is applied as late as September 20 at New Brunswick. To determine the necessity of removing the dirt and the crystals, pull the dirt away from several trees in the orchard before the ground freezes and if no crystals are seen or a strong odor detected it will be unnecessary to remove the dirt.

Our present information indicates that the best time to make an application of paradichlorobenzene is the last week in August or the first ten days in

September, August 25 to September 10. In the average season applications made at this time will kill the borers in the trees, prevent further infestation that season, and give ample time for all or a greater portion of the paradichlorobenzene to evaporate before the ground becomes cold or freezes.

5. METHOD OF APPLICATION

We recommend on the basis of our experiments the following method of application. Prepare the soil for treatment about the base of the tree by removing the grass, weeds and other refuse for a distance of at least one foot. Then make the soil smooth and level for a distance of 6 inches from the tree (pl. 1, fig. 1). Do not dig into the surface crust any more than is necessary, for by so doing numerous large air spaces will be made which are probably undesirable. In case a considerable amount of gum is present about the base of the tree (pl. 1, fig. 4) it is best to remove the bulk of it. It is also advisable to have the surface of the soil where the paradichlorobenzene is applied, level with the highest point on the tree where a considerable amount of gum, containing sawdust-like particles, is exuding. The greatest number of larvae will be killed if this point is observed. We know that the gas coming from the crystals is heavier than air; consequently, if the paradichlorobenzene is placed below some of the larvae in the tree the amount of gas going up from the material is apt to be of insufficient strength to kill the borers above the point of application. In measuring out $\frac{3}{4}$ or 1 ounce of paradichlorobenzene use some vessel which holds, when filled, the required amount. A short wide-mouth bottle, a tin or wooden pill box may answer the purpose.

The paradichlorobenzene is evenly distributed in a narrow continuous circular band on the soil about the tree approximately two inches from the trunk (pl. 1, fig. 2). The band should be about 1 inch wide and no crystals should be closer than 1 inch from the trunk (or large roots), otherwise injury may take place (pl. 1, fig. 5). In case large roots are near the surface of the soil at least one or more inches of dirt should be placed above them before applying the paradichlorobenzene. If the crystals are placed several inches (4 or more) from the tree (pl. 1, fig. 6) the effectiveness of the insecticide is apt to be materially reduced. This is particularly true if the soil is wet.

After the paradichlorobenzene is properly distributed place several shovels of earth (free of weeds, grass, large stones or other refuse) over the "death ring" of paradichlorobenzene and compact it into a cone-shaped pile with the back of the shovel or some other tool (pl. 1, fig. 3). The first shovel of earth placed on top of the treatment should be finely divided and carefully poured upon the paradichlorobenzene. Forcefully throwing the first shovel of earth directly onto the ring of crystals is apt to push some of the material against the tree. This may cause injury. After we learn more about this product we may find that some of the above points may be changed or adherence to some of them may be unnecessary.

6. SOIL CONDITIONS

Our experiments to date indicate that soil texture does not have much influence upon the rate of evaporation. This is particularly true if the soil is dry. Also, soil acidity or alkalinity does not seem materially to affect the rate of evaporation of paradichlorobenzene. The details of the experiments employed in determining the above points are omitted.

Soil moisture

The amount of moisture in the soil has a direct influence on the rate of evaporation of paradichlorobenzene. In our laboratory experiments with four kinds of soil, Penn loam, clay loam, sandy loam and white sand, 1 gm. of paradichlorobenzene evaporated completely in 15 to 16 days when embedded (2 inches deep in 6-inch flower pots) in dry soil and kept at an average temperature of 70°F., while in a similar series where the four soils were kept moist by adding 50 cc. of water each day the 1 gm. of material required four to seven times as many days to disappear completely, the maximum time being 113 days. In other laboratory experiments with varying degrees of soil moisture there was a consistent ratio between the rate of evaporation of the paradichlorobenzene and the amount of water present in the soil.

In several orchard experiments it was noted that moist soils retained the paradichlorobenzene for a greater period of time than dry soils. In one orchard a number of trees were treated with 1 ounce of paradichlorobenzene in June. Some of these trees were on high ground and others on low ground. When the dirt was removed in October the crystals were present and easy to find about the trees in the low ground where the soil had been moist or wet all the season, while no crystals and no odor was present in the soil about the trees on high ground where the soil was much drier. Generally speaking, it requires 6 to 8 weeks for the crystals to evaporate completely and for the gas to disappear when the soil temperature is 60°F. or higher and the soil moisture is low.

We know that paradichlorobenzene is practically insoluble in water; consequently, if evaporation is to take place in the soil it is dependent upon the amount of air in the soil. Water-laden soils possess few or no continuous air spaces, while dry soils are porous containing numerous small air spaces. An excessive amount of water in the soil fills all of the air spaces and thus acts as a barrier to the fumes arising from the paradichlorobenzene.

If the amount of water present in the soil influences the rate of evaporation and distribution of the gas, then one would expect to find that the moisture content of the soil influences the toxicity of paradichlorobenzene as an insecticide for the peach-tree borer. A few experiments have been conducted to determine this point and the results show that fumigation under different degrees of soil moisture produces a decided difference in the percentage of dead larvae when the borers are subjected to short exposures. Seven to ten-

day treatments with $\frac{1}{2}$ ounce in wet (saturated) soils were 40 to 50 per cent less effective than in semi-moist soils where the water content was 25 to 50 per cent less than that of the wet soils (table 3, exp. 1-6).

In the laboratory experiment described below the moisture content of the soil had a decided influence on the distribution of the gas. Two large metal containers (open at the top and the bottom) 12 by 12 by 10 inches were filled with dry sand. The sand in one of these was saturated with water. One-half ounce of paradichlorobenzene was embedded (2 inches deep) in the center in a 2-inch circle in each container. Pieces of fresh peach bark (1 by 3 inches) containing newly hatched larvae (2 to 9 larvae in each piece of bark) were

TABLE 3

Influence of soil moisture and temperature on paradichlorobenzene treatments for the control of the peach-tree borer; trees 4 years of age; moisture experiments 1-6; temperature experiments 7-11

NUMBER	NUMBER OF TREES	AMOUNT APPLIED	SOIL WATER	DATE TREATED AND SOIL TEMPERATURE	DATE "WORMED" AND SOIL TEMPERATURE	LARVAE PER TREE		PER CENT DEAD
						Alive	Dead	
		oz.	per cent					
1	*5 (W)	0.5	20±	October 26, 58° F.	November 5, 44° F.	4.4	1.4	24
2	*5 (W)	0.5	15—	October 26, 58° F.	November 5, 44° F.	2.8	4.6	62
3	5 (B)	0.5	30	October 14, 58° F.	October 21, 58° F.	4.4	0.6	11
4	10 (B)	0.5	20	October 14, 58° F.	October 21, 58° F.	1.7	4.8	74
5	*5 (W)	1.0	Wet	June 28	October 19	1.4		
6	*5 (W)	1.0	Dry	June 28	October 19	3.0		
7	10 (W)	0.5	10-15	October 19, 60° F.	October 26, 58° F.	2.0	6.5	76
8	10 (W)	0.5	10-17	November 1, 55° F.	November 11, 44° F.	8.4	1.8	17
9	10 (B)	0.5	17—	November 1, 45° F.	November 8, 48° F.	3.0	0.0	0
10	10 (B)	0.5	17—	November 8, 48° F.	November 22, 41° F.	2.0	0.3	13
11	10 (B)	0.5	17—	November 1, 45° F.	November 22, 41° F.	1.0	0.9	47

* One hundred eggs placed on each tree.

(W) Wolpert Farm, New Brunswick; Sassafras loam soil.

(B) Bound Brook Nurseries, Penn loam soil.

embedded in the soil at intervals of 1, 2, 3 and 4 inches in two directions from the paradichlorobenzene. Other pieces of bark containing larvae were placed in dry and wet sand in flower pots free of paradichlorobenzene. These served as checks. After 3 days' exposure all of the pieces of bark were examined. In the checks all of the larvae were alive. In the container filled with dry sand all of the larvae were dead, while in the container filled with wet sand 10 per cent of the larvae were alive in the pieces of bark 3 inches from the paradichlorobenzene and 40 per cent were alive in the pieces of bark 4 inches from the insecticide. All other larvae in all pieces of the bark in this container were dead. This experiment shows how water may act as a barrier to the gas.

In prolonged treatments made in wet soils the final results may prove to be just as effective as in semi-moist soils. This is expected if the soil becomes somewhat dry sometime after the applications are made and if the soil temperature is 55–60°F. or higher. Further investigation is needed to determine the influence of moisture in long exposures.

Soil temperature

The temperature of the soil also influences the rate of evaporation of paradichlorobenzene. In a number of laboratory experiments where 1 gm. of paradichlorobenzene was embedded (2 inches deep) in each of three flower pots containing dry sand and kept at average temperatures of 68°, 50° and 42°F. for periods of 10 and 20 days, the rate of evaporation in the pot kept at 68°F. was four times as fast as in the pot kept at 42°F. and approximately two times as fast as in the pot averaging 50°F. About the same rate of evaporation held for crystals exposed to open air for 24 hours with the temperatures averaging 70°, 52° and 42°F. Briefly stated our experiments show that high temperatures produce greater rates of evaporation of paradichlorobenzene than low temperatures. If the rate of evaporation varies with the temperature, then one would expect to find that paradichlorobenzene is less effective as an insecticide at low temperatures than at high temperatures.

This effect is shown in a few experiments with short exposures conducted this past season. Table 3 on moisture and temperature experiments shows in one series (exp. 9–11) that 47 per cent of the larvae were killed in 3 weeks when the temperature was 48°F. or less, and in another series (exp. 8) only 17 per cent of the borers were killed by 10 days' exposure at temperatures between 55° to 44°F., while 76 per cent were killed with 7 days' exposure (exp. 7) at 60–58°F. Longer exposures at temperatures of 55° or less might kill a larger percentage of the worms. Further investigation is needed to determine this point.

The above ineffectiveness of paradichlorobenzene at low temperatures probably is not altogether due to the fact that the paradichlorobenzene evaporates more slowly at low temperatures. It is in part due to the fact that at soil temperatures under 55°F. the larvae are inactive and consequently their oxygen requirements are lower.

If low temperatures do not give immediate and effective results, then the question arises, what is the minimum temperature which produces an immediate and effective kill? Our experiments show that the best results with short exposures of paradichlorobenzene are obtained when the soil temperature at a depth of 4 inches under the peach trees registers 60°F. or higher. Fairly satisfactory results have been obtained at temperatures ranging between 55° to 60°F. Below 55°F. the effectiveness and the rapidity of kill with paradichlorobenzene is materially reduced. This is particularly true in short exposures.

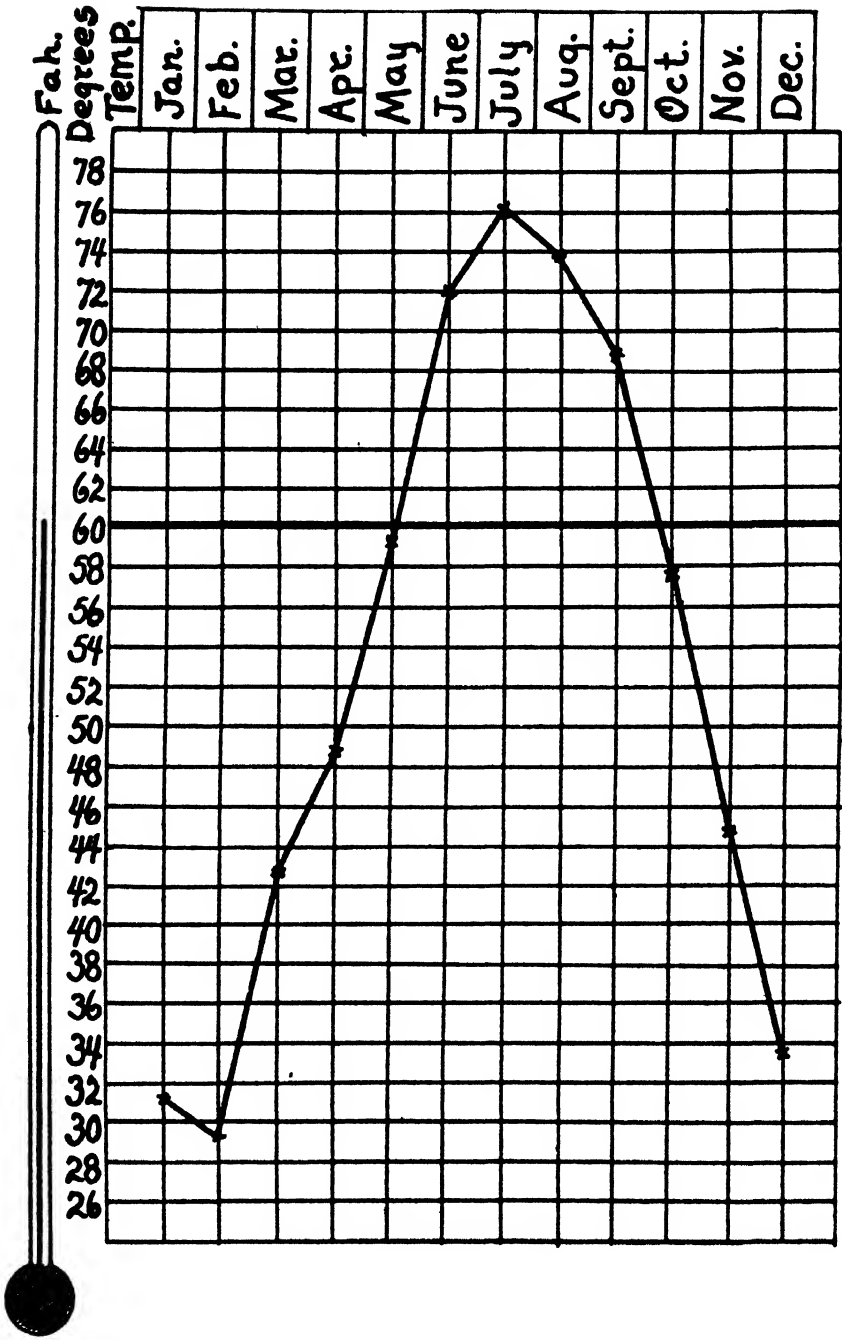


FIG. 1

The plotted line shows the monthly mean soil temperature at six inches for 1898, 1901 and 1902 at New Brunswick, N. J.

The chart with a plotted line shows the monthly average for 1898, 1901 and 1902 of the mean soil temperatures at 6 inches at New Brunswick, N. J. If 60°F. proves to be the minimum temperature for the most satisfactory results with paradichlorobenzene in the control of the peach-tree borer, then at New Brunswick, N. J., an immediate and effective kill of the borer will take place when applications are made between May 15 and October 1, provided the soil moisture is not too great. Earlier or later applications for the average season will not give immediate and effective results because the soil is too cold (fig. 1).

7. COST

Paradichlorobenzene may be purchased from several firms in the United States for 15 to 30 cents a pound, depending largely upon the amount purchased. In several orchards we kept account of the cost of treating trees of various ages under different orchard conditions. Under very unfavorable orchard conditions the greatest cost of a 1-ounce treatment with the material at 20 cents a pound was approximately 4 cents a tree, while in other orchards where 1-ounce applications were more easily made the cost averaged about 3 cents a tree. In one orchard of 800 3- and 4-year-old trees a $\frac{1}{2}$ -ounce application, including the removal of the dirt to the ground level in 7 to 10 days, cost 3.5 cents a tree.

8. OTHER EXPERIMENTS

The rate of evaporation of paradichlorobenzene is also somewhat dependent upon the size of the crystals. Various experiments, indoors and outdoors, above and below ground, were conducted with crystals of varying sizes, particularly 3 to 5, 5 to 10, 10 to 20 and 20 meshes (or smaller) to the inch (table 4). One gram or smaller amounts of crystals were placed in glass dishes and exposed to the open air or placed in dry sand (2 inches deep) under inverted glass dishes. The crystals were separated as much as possible in order that the surface of each should be exposed to the air. The rate of evaporation under the above conditions shows conclusively that the large crystals evaporated much more slowly than the small ones. This is expected because a small crystal has a proportionally greater surface than a large crystal. In similar experiments where the crystals were piled on top of each other the rate of evaporation of the crystals of different sizes was more nearly uniform. In field practice the crystals are piled on top of each other, more or less, when placed about a tree, consequently one would not expect a great variation in the rate of evaporation of small and large crystals. This appears to be true where the crystals range from 3- to 20-mesh.

The insecticidal value of large crystals appears to be as great as that of the small crystals. Until we have further information on the influence of the size of the crystals it is believed that they should be 10-mesh or finer. In case the material is lumpy it should be passed through a wire-screen sieve made out

of ordinary window screening or some screen having 10 or more openings to the inch. Finely divided crystals are easy to distribute evenly, and we know that good results may be obtained if they are used.

Some laboratory and field experiments show that full grown larvae of the peach-tree borer in cocoons preparing to pupate and pupae in cocoons may be killed with paradichlorobenzene. The larvae in the cocoons seem to be more susceptible than the pupae. During the fore-part of August, 1920, two lots of 10 and 15 pupae were embedded in sandy loam soil in flower pots (5 inches in diameter) and exposed to weather conditions. Ten and 15 pupae were placed in the center of two pots and 5 gm. of paradichlorobenzene was sprinkled in a circle about them. No crystals were closer than 1 inch from the pupae. Two other pots possessing 10 and 15 pupae served as checks and received no paradichlorobenzene. The pupae and the paradichlorobenzene in all four pots were covered with 1 inch of soil. In September the pupae were examined and 21 out of 25 pupae had emerged in the untreated pots while 1 out of 25 emerged in the treated pots.

TABLE 4

Table showing the number of hours required to evaporate completely crystals of paradichlorobenzene of four sizes

CONDITIONS	3 to 5-mesh	5 to 10-mesh	10 to 20-mesh	20-mesh
	hours	hours	hours	hours
One gram of crystals placed in glass dish; exposed to room air and average temperature of 80° F.	48	26	18	12
One-fourth gram of crystals embedded 2 inches in dry sand in flower pots (5 inches in diameter), exposed to room air and average temperature 70 to 80° F.	190	153	126	105

Another experiment with pupae and paradichlorobenzene was started on August 25 outdoors in the ground at the laboratory. Individual pupae (20 altogether) were embedded 1 inch deep in sandy loam soil at intervals of 1, 2, 3, 4 and 5 inches away from a 2-inch circle of $\frac{1}{2}$ ounce of paradichlorobenzene embedded 1 inch in the ground. There were four lines of pupae at right angles to each other, making a total of 4 pupae at each distance. On September 20 the experiment was observed and 18 of the 20 pupae had emerged. One pupa at 1 inch and one at 2 inches failed to emerge. This experiment indicates that the pupae were not affected by the paradichlorobenzene. There seems to be a decided difference in the results of the above experiments with the pupae.

It is possible that the fumes of the gas were much stronger in the flower pots than in the ground outdoors. Furthermore, in the flower pots the pupae were inside of a circle of paradichlorobenzene. The fumes are undoubtedly much stronger inside a small circle than on the outside of a mass. Unfortunately the moisture conditions of the soil in the above experiments were

unknown. Probably the soil in the pots exposed to the wind and sunlight was much drier at times during August than the ground soil. Further experiments are needed to determine the influence of paradichlorobenzene on the pupae of the peach-tree borer.

Other experiments have been conducted on other phases of soil fumigation with paradichlorobenzene, but at this time the information is insufficient to report.

CONCLUSIONS

Paradichlorobenzene gives considerable promise of becoming a valuable and an important insecticide for the control of the peach-tree borer. The use of paradichlorobenzene for the control of the peach-tree borer is still in the experimental stage; however, we feel that sufficient information has been secured to warrant an announcement concerning its possibilities as an insecticide for the most destructive peach-tree pest. It is suggested that peach growers try out this material for a year or two on a small block of trees 6 years of age or older before treating their entire planting of old trees.

Paradichlorobenzene for the peach-tree borer is not a "fool-proof" remedy, consequently there are certain points in its use which must be carefully followed. Briefly stated, $\frac{3}{4}$ to 1 ounce of paradichlorobenzene will kill 90 to 100 per cent of the borers if the soil temperature is 55 to 60°F. or higher, and the soil is not too wet. It can be used with a considerable margin of safety on trees 6 years of age or older. In making applications the finely divided crystals are evenly distributed in a narrow continuous circular band on smooth ground about the base of the tree. The band should be approximately 2 inches from the tree and no crystals should be closer than 1 inch from the tree. In case there are indications of borers in the tree 1 to 6 inches above the ground, the best results are obtained if the soil is mounded about the tree so that the upper level of the soil is even with the highest point where the gum, containing saw-dust-like particles, is exuding from the tree. The application should then be made on the new soil level. After the paradichlorobenzene is properly distributed place several shovels of dirt, free of weeds, grass, large stones, sticks, etc., over the "death ring" of crystals and pack down the dirt with the back of a shovel or some other tool. For New Jersey conditions the best time of the year to make applications is during the last week in August or the first 10 days in September, August 25 to September 10.

ACKNOWLEDGMENT

The writer is indebted to a number of owners of peach orchards in New Jersey for coöperation in this investigation. He also wishes to thank Dr. T. J. Headlee, Dr. J. G. Lipman and other individuals who have given many valuable suggestions and information pertaining to the problem.

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PLATE 1

FIG. 1. The soil about the peach tree made smooth and ready for treatment.

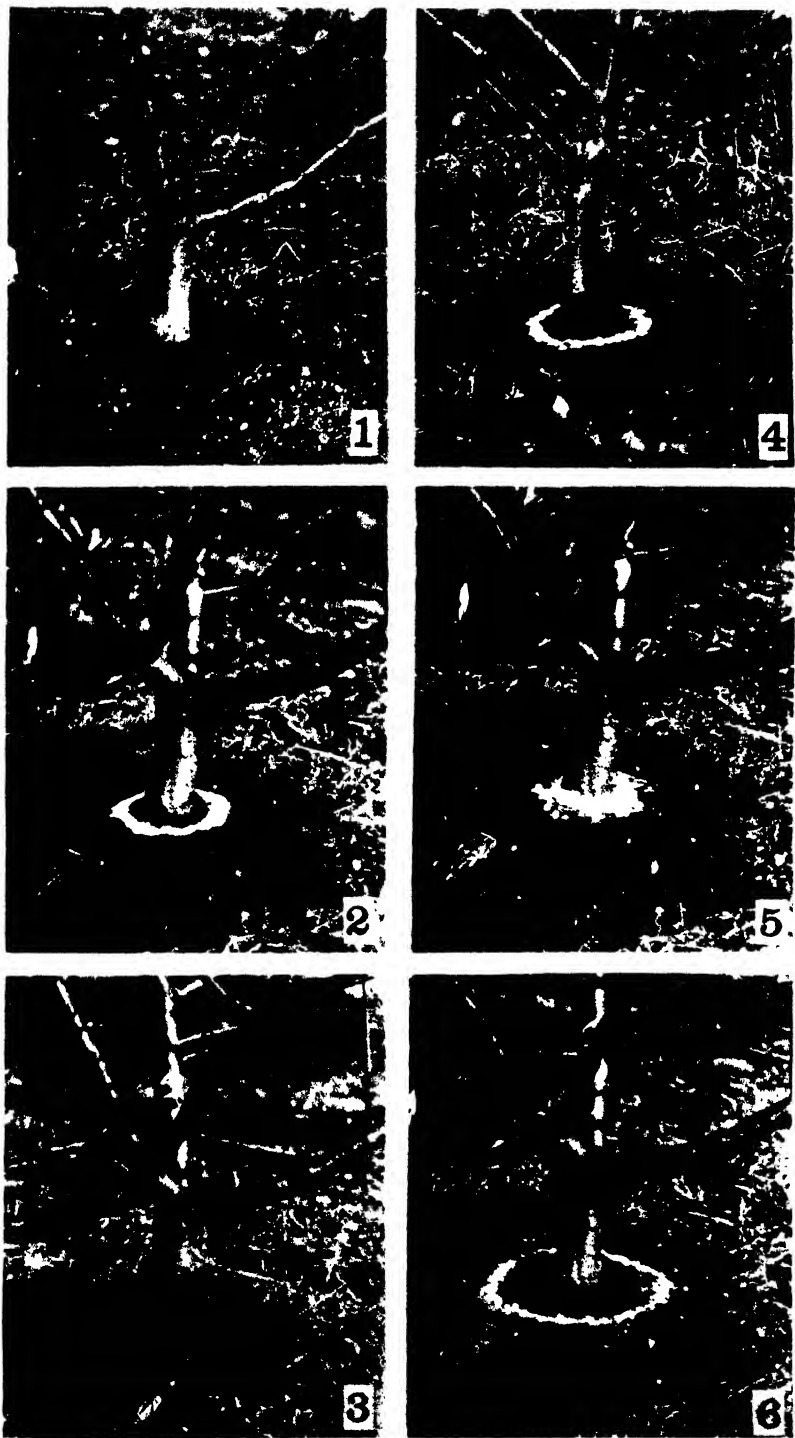
FIG. 2. One ounce of finely divided paradichlorobenzene placed on the soil about the tree in a continuous narrow band approximately 2 inches from the tree; correctly applied.

FIG. 3. Dirt free of grass, large stones, sticks, etc. placed on top of the paradichlorobenzene 4 to 6 inches deep and packed down.

FIG. 4. Paradichlorobenzene incorrectly applied; note the gum exuding from the tree 4 inches above the soil level; dirt should have been placed about the tree to a level equal with the highest point where the gum is exuding before the application was made.

FIG. 5. Paradichlorobenzene incorrectly applied; if the material is placed against the trunk serious injury or death may take place; all crystals should be at least one inch from the tree; also note the large lumps; these are undesirable.

FIG. 6. Paradichlorobenzene incorrectly applied; in this figure the material is placed too far away (6 inches) from the tree to be effective as a killing agent for all of the borers.



THE EFFECT OF CONTINUOUS CROPPING UPON THE MAJOR SOIL NUTRIENTS

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Thirteen samples of California soils from sections of the state as widely separated as the grain lands of the northern Sacramento Valley and the orange groves of Riverside were collected and brought to the Agricultural Experiment Station at Berkeley in 1915. For six seasons these have been the basis for a series of intensive studies of the changes that occur in soil nutrients as the result of the growth of successive crops of cereals.

The first criterion in the choice of these samples was similarity of texture. Six of the thirteen were silty clay loams, collected from different areas, but all, according to the maps of the United States Bureau of Soils, members of the same soil series. These six, then, displayed similarity not only in the texture of the soil, but in their color and general appearance as well. The remaining seven were fine sandy loams. The members of this group were chosen so that each of them represented a different soil series, derived from a different geological formation. While their texture was similar, there was considerable variation in their color and general appearance.

In order to avoid the differing seasonal and climatic influences to which they would have been subjected in their normal environments, the soils were brought to Berkeley in 2-ton samples. Here each sample was divided between duplicate containers holding approximately 1800 pounds.

For the first season, all the containers were planted to Beldi barley. In the succeeding years, one container of each soil has been cropped with this same grain, while the other has been left unplanted. During this time the soils have been quite removed from the leaching effect of rainfall or of excessive irrigation. During the growing season the moisture content of the planted and unplanted containers alike has been maintained at the optimum by the daily addition of distilled water.

We have had available then, under the condition of continuous cropping and that of continuous fallowing, these two sets of soils, of which the members of one set were united by the closest possible relationship, while the members of the other were of exceedingly diverse origin. It has been possible, therefore, to draw between the members of each set, conclusions as to chemical differences in composition and in crop production.

Elsewhere there have been reported the earlier portions of the work dealing with seasonal studies of the water extract (4) and of the freezing-point depres-

sion (2), as well as comparisons of the chemical composition (1). For the purposes of this article it will be sufficient to state that differences of productivity in either set of soils were not associated with corresponding differences in the total chemical composition as estimated by any of the usual methods; that the most adequate criteria of such differences appeared to be the seasonal studies of the water extract, reflecting as they do the changes in the soil solution, and the expression of the total of these changes by the freezing-point depression of the soil.

By means of these methods of procedure striking differences were observed between the planted and unplanted soils, and also between the individual soils. It was clearly shown that the water-soluble nitrates, calcium, potassium, and magnesium present in the soil were notably reduced by the growing crop. Great dissimilarities in the phosphate content of the different soils were observed, although the water-soluble phosphates did not at first show appreciable changes resulting from the growth of the crop.

The details of these results are given in the papers previously issued.

Under continuous cropping, these soils, as might be expected, have notably decreased in crop production. They have not yet reached a constant level. The average decrease of the total crop amounts to 34.9 per cent, that of the straw to 35.2 per cent, and that of the grain to 34.4 per cent.

The study of the soil nutrients in the continuously planted soils has developed certain interesting facts. Observations of the phosphates, during the earlier seasons, did not reveal an appreciable reduction of this constituent as a result of the growing of a crop. But a comparison of the results of five years discloses the fact that in five of the seven sandy loams there has been a reduction of 30 per cent or more in the content of soluble phosphates, while only two of the silty clay loams have begun to show a decrease in this nutrient. There is no indication, so far, that the lowering of the phosphate content accounts for the first decrease in the crop yield; for most of the soils whose soluble phosphates have been reduced are at the present time producing the best crops both of grain and of straw.

Determinations made before the soils were planted showed that the fine sandy loams contained from 0.05 to 0.09 per cent, and the silty clay loams from 0.13 to 0.18 per cent of total nitrogen. Of this nutrient there has been a continual loss. At the close of the season of 1919 all of the soils, both planted and fallow, showed a reduction of from 14 to 38 per cent of their original total nitrogen content.

Investigations of nitrogen losses have generally been made under humid conditions, where drainage has been a large factor in the disappearance of nitrates. Shutt (3) and a few other workers have studied the decrease of nitrogen in prairie soils subjected to little excess rainfall. Their results show that the crop removed only one-third of the nitrogen lost.

In the experiment here described, where the soils have never been subjected to leaching from rainfall or over-irrigation, each season's crop removed

about 17 gm. of nitrogen from each container, while the annual loss from the same soil amounted to about 60 gm. It should be noted, also, that the nitrogen loss from the uncropped soils was almost as great as was that from the planted containers. This constitutes an interesting example of the decrease of our most valuable soil constituent through the ordinary processes of cultivation and tillage. It illustrates, too, the decrease in soil productivity resultant upon a one-crop system of grain farming.

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HYDROGEN-ION CONCENTRATION RELATIONS IN A THREE-SALT SOLUTION

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INTRODUCTION

The interest of a considerable number of workers, using water-cultures, in solving various phases of the salt-requirements of plants or allied problems, has centered itself in part on attempting to find a desirable control solution. Such a solution should be as simple as possible and defined in such a way as to be duplicated readily by other workers. It would be defined physiologically, in terms of the plant's response; chemically, by a statement of the molecular proportions of its component salts; and physically, by the magnitude of its osmotic properties. A still more complete statement would include the degree of true acidity or alkalinity existing in the solution, as determined by the hydrogen-ion concentration.

Knop's (8), Totttingham's (18), and Shive's (14) 3-salt solutions, have all been recognized as standard solutions. Little is known, however, concerning their true reaction. It was considered worth while to repeat Shive's work with the purpose of determining the part played by the hydrogen-ion concentration in the 36 different sets of salt proportions which he employed.

The publication by the Special Committee on Salt Requirements of Representative Agricultural Plants (16) resulted in our modifying our original purpose to the extent of using instead of the 36 sets of salt proportions having an osmotic concentration of 1.75 atmospheres, the 21 sets of salt proportions of type I with an osmotic concentration of 1.00 atmosphere.

The importance of the true reaction of a culture medium as affecting its biological activities is well recognized. However, only relatively few studies have been made of the direct physiological influence of reaction as measured by the hydrogen-ion concentration upon plants grown in solution-culture.

Toole and Totttingham (17) found a correlation between the weight yield of tops and the hydrogen-ion concentration of the solution—"these two values varying in opposite directions." Hoagland (5) carried out solution-culture experiments in which the concentration, composition and reaction were under control. Intensity of reaction was determined by means of the H-electrode. Salter and McIlvaine (12), more recently, have determined the effect of reaction upon the growth and germination of seeds of wheat, soybean,

corn and alfalfa. A reaction of 2.16 pH was fatal to the seedlings of all the plants, while a reaction of 7.71 pH depressed the growth of all except the corn seedlings.

MATERIALS AND METHODS

The methods used in the preparation of the culture solutions, the germination of the seed and the treatment of the plants throughout the growth period, were those recommended by the Committee on Salt Requirements of Repre-

TABLE 1

Partial volume-molecular concentrations and molecular proportions of KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, and MgSO_4 in the 21 solutions, differing by increments of one-eighth in the salt proportions, but all having an osmotic value of approximately 1.00 atmosphere at 25°C.

SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME-MOLECULAR CONCENTRATIONS		
	KH_2PO_4	$\text{Ca}(\text{NO}_3)_2$	MgSO_4	KH_2PO_4	$\text{Ca}(\text{NO}_3)_2$	MgSO_4
IR_1S_1	1	1	6	0.0027	0.0027	0.0161
S_2	1	2	5	0.0025	0.0049	0.0123
S_3	1	3	4	0.0024	0.0071	0.0094
S_4	1	4	3	0.0022	0.0089	0.0067
S_5	1	5	2	0.0022	0.0108	0.0043
S_6	1	6	1	0.0020	0.0122	0.0020
R_2S_1	2	1	5	0.0053	0.0027	0.0132
S_2	2	2	4	0.0049	0.0049	0.0099
S_3	2	3	3	0.0047	0.0071	0.0071
S_4	2	4	2	0.0045	0.0090	0.0045
S_5	2	5	1	0.0041	0.0104	0.0021
R_3S_1	3	1	4	0.0076	0.0025	0.0101
S_2	3	2	3	0.0072	0.0048	0.0072
S_3	3	3	2	0.0068	0.0068	0.0045
S_4	3	4	1	0.0065	0.0086	0.0021
R_4S_1	4	1	3	0.0099	0.0025	0.0074
S_2	4	2	2	0.0094	0.0047	0.0047
S_3	4	3	1	0.0090	0.0068	0.0022
R_5S_1	5	1	2	0.0123	0.0024	0.0049
S_2	5	2	1	0.0118	0.0047	0.0023
R_6S_1	6	1	1	0.0145	0.0024	0.0024
Shive's.....	3.77	1.09	3.14	0.0180	0.0052	0.0150
K^*				0.0044	0.0145	0.0050
T^\dagger				0.0108	0.0101	0.0081

(In addition to the three salts listed above, Knop's, K, and Tottingham's, T, solutions also contain potassium nitrate in the concentrations as given.)

* K — KNO_3 = 0.0059.

† T — KNO_3 = 0.0034.

sentative Agricultural Plants (16). Table 1 gives the molecular proportions and the volume-molecular concentration of the solutions used. The relation of the 21 solutions of type I to each other may be shown readily by the use of the triangle diagram (fig. 1) described in various publications.¹ In addition to the 21 sets of salt proportions varying in increments of one-eighth of the total volume-molecular concentration, a single culture in distilled water was conducted during each of the three time-periods as well as triplicate cultures of Shive's "best," R_5C_2 , having an osmotic concentration of 1.75

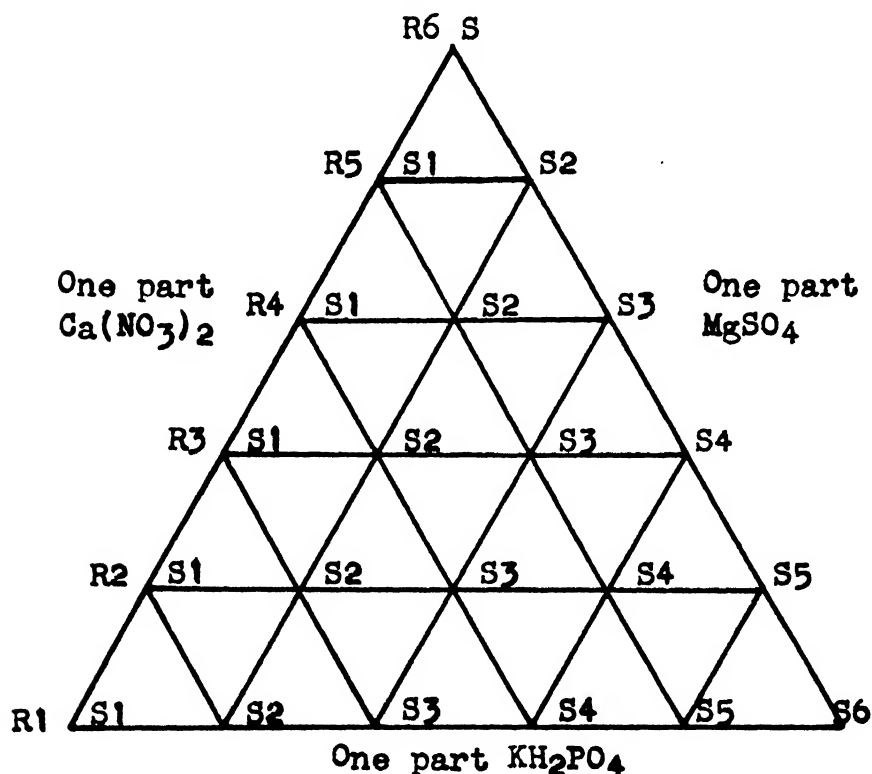


FIG. 1. DIAGRAM SHOWING SOLUTION NUMBERS AND VOLUME-MOLECULAR PROPORTIONS OF THE THREE SALTS

atmospheres. For purposes of comparison, there were conducted during the latter two time-periods, duplicate cultures of Knop's solution (designated as K in all of the tables) and Tottingham's best solution (designated as T in all of the tables), each having an osmotic concentration of 1.75 atmospheres (14).

The wheat plants used in this series of experiments belonged to the Fulcaster variety. The seed was obtained from the United States Department of Agriculture. The method of germination used was that described by the Plan of the Committee on Salt Requirements, etc. (16). Seedlings of uniform

¹ For the application of the triangle diagram to studies in plant nutrition, see Schreiner and Skinner (13), Tottingham (18), Shive (14) and Johnson (7).

height were selected and threaded through holes in paraffined corks, prepared as suggested by Tottingham (18). Each culture bottle contained 6 seedlings. The jars used to contain the culture solutions were of white flint glass similar in dimensions to those used by Shive, but having a capacity of slightly more than 210 cc. Before being used, the jars and all the glassware used in the experiments was cleansed with chromic acid cleaning fluid, steamed in the autoclave, treated with alkali, followed with cleaning fluid, rinsed several times with tap water and finally four times with distilled water.

To protect the roots in the culture jars from the light and excessive absorption of heat, the jars were wrapped in paper jackets which were black inside and white outside. These jackets or wrappers were prepared as described by Shive (14).

The solutions in the culture jars were replaced at the end of each $3\frac{1}{2}$ days by fresh solutions of similar volume. Hence the solutions were renewed nine times during the growth period of 35 days. As the rate of absorption is approximately the same as the rate of transpiration in wheat (10), we have in the amount of solution absorbed a measure of the transpiration; these data are recorded elsewhere as transpiration.

Since it was impossible to control the external conditions under which the cultures were to be conducted, the alternative was to submit all the plants to these conditions in an approximately similar way. This was accomplished practically by placing the cultures on a slowly rotating table; the cultures in a given circle on the table were subjected to the same variations in the surroundings. As the solutions were always used in duplicate there were two rows of circles on the table. The outside row is designated throughout this paper as series A, and the inner row, as series B. Thus the two circles or series of cultures for a given growth period had slightly different aerial surroundings, although the different cultures of the same circle were comparable. The rotating table used in this work was 4 feet in diameter and was attached to a ball-bearing base. The table was rotated by a small $\frac{1}{2}$ -horsepower motor, belted to a reducing gear, which, in turn, was belted to the table. The table made a complete revolution every 4 minutes.

As it was appreciated that seasonal variations influence the physiological condition of the plant and hence the growth rate—the culture series were conducted in duplicate through three different time-periods of the year. It was hoped by this means to eliminate seasonal variation and to arrive, at least approximately, at the physiological properties of the solutions as determined by the chemicals alone. The first duplicate series was conducted from July 1 to August 5, 1919. The second duplicate series was conducted from November 23 to December 28, 1919; and the third from January 21 to February 25, 1920. The length of each time-period was 35 days. The period from July 1 to August 5 is characterized throughout this paper as the “first time-period,” the period from November 23 to December 28, as the “second time-period;” and the period from January 21 to February 25 as the “third time-period.”

Determination of hydrogen-ion concentration

The initial reaction of the culture solutions was determined and also the reaction at the end of each 3½-day interval, when the solutions were renewed. The hydrogen-ion concentrations of the solutions employed in the experiments recorded here were determined colorimetrically according to the method of Clark and Lubs (1). The recommendations of these authors in the purification of the salts for the buffer mixtures, were followed carefully. Although it is desirable to check the values of the buffer mixtures against the hydrogen electrode, it is not altogether necessary when relative rather than absolute values are desired. Clark and Lubs have pointed out that the absolute accuracy of the buffer mixtures is far within the experimental error that occurs in determining differences of color value between an unknown and the standards. Gillespie and Hurst (3, 4), using buffer mixtures prepared similarly in their determinations on soils, checked the mixtures electrometrically. They confirm the values assigned by Clark and Lubs and the accuracy of the composition of the buffer mixtures, adding that "if the substances used are pure this is unnecessary."

RESULTS

Aerial conditions

Records were obtained for: (a) air temperature; (b) relative humidity; (c) evaporating power of the air; (d) intensity of the absorbed radiant energy; and (e) duration of sunshine.

(a) Temperature changes and changes in the relative humidity were recorded on a hygro-thermograph placed near the rotating table. The maximum and minimum changes of temperature were recorded daily from the thermograph sheet and the mean temperature calculated. The data of these changes are expressed in terms of the Centigrade scale.

(b) The changes in the relative moisture-holding capacity of the air were recorded daily, and show maximum, minimum, and the mean expressed as relative humidity. The hygrograph was standardized at the beginning of each time-period by means of a sling psychrometer and the relative humidity determined by reference to Marvin's (11) psychrometric tables.

(c) The evaporating power of the air was measured by means of white and black standardized spherical porous cup atmometers [Livingston (9)]. The atmometers were weighed and cleaned weekly. Readings were corrected by multiplying by the coefficient of correction of the cup used.

(d) The intensity of the absorbed radiant energy [Livingston (9)] is expressed as the difference in the losses between the black cup and the white cup. Both white and black cups were placed on the innermost circle on the rotating table along with one of the control solutions and the culture in distilled water.

(e) The duration of sunshine expressed in hours, was obtained from the records of the Marvin Sunshine Recorder of the United States Weather Bureau Station located about 300 yards from the greenhouse in which these experiments were performed.

A summary of the aerial conditions for each time-period is given in table 2.

TABLE 2
Weekly averages of aerial conditions for the three time-periods

DATE, WEEK ENDING	ATMOMETRIC DATA (CORRECTED)			SUNSHINE*	RELATIVE HUMIDITY			TEMPERATURE		
	Loss white cup	Loss black cup	Difference		Average maximum	Average minimum	Mean	Average maximum	Average minimum	Mean
Time-period: July 1 to August 5, 1919										
	gm.	gm.	gm.	hours	per cent	per cent	per cent	°C.	°C.	°C.
July 8.....	82.6	153.6	70.9	100.6	79.5	38.1	58.8	33.2	14.0	23.6
July 15.....	113.5	130.9	17.5	49.4	80.7	37.5	59.1	29.2	11.3	20.3
July 22.....	76.0	98.2	22.2	69.5	80.8	41.1	60.9	32.0	15.1	23.5
July 29.....	109.1	130.7	21.6	68.4	79.8	43.4	61.6	32.7	14.1	23.4
August 5.....	83.4	112.7	29.3	58.1	80.1	39.2	59.6	27.0	11.5	19.3
Total.....	464.6	626.1	161.5	346.0						
Daily average.....	13.3	17.9	4.6	9.8	80.1	39.8	59.9	30.8	13.2	22.0
Time-period: November 23 to December 28, 1919										
November 30.....	96.5	106.9	10.4	8.5	75.5	56.7	66.1	22.0	14.6	18.3
December 7.....	114.5	137.7	23.3	17.7	76.5	45.1	60.8	25.3	11.6	18.5
December 14.....	112.5	123.2	10.7	14.6	76.2	50.2	63.2	23.6	13.1	18.4
December 21.....	109.1	125.0	15.8	31.3	75.2	44.0	59.6	24.8	11.8	18.3
December 28.....	109.6	120.6	11.0	16.7	70.2	37.4	53.8	28.0	12.7	20.4
Total.....	542.2	613.4	71.2	88.8						
Daily average.....	15.5	17.5	2.0	2.4	74.7	46.7	60.7	24.7	12.8	18.8
Time-period: January 21 to February 25 1920										
January 28.....	117.4	139.0	21.7	24.9	67.7	41.7	54.7	27.7	14.6	21.2
February 4.....	126.1	143.4	17.3	26.3	64.7	37.5	51.1	27.8	14.1	21.0
February 15.....	168.9	193.3	24.4	21.5	68.0	41.6	54.8	28.2	14.9	21.6
February 18.....	50.9	58.3	7.3	11.0	68.3	42.3	55.3	28.1	15.2	21.7
February 25.....	114.9	143.5	28.6	36.4	66.8	34.5	50.7	29.7	14.9	22.3
Total.....	574.1	677.5	99.4	120.1						
Daily average.....	16.5	19.4	2.8	3.3	67.1	39.5	53.3	28.3	14.7	21.5

* We are indebted to Mr. Morgan R. Sanford, of the U. S. Weather Bureau Station, for the figures from which the data of this column were computed.

Fresh weight of tops

All of the plant cut off just above the grain was considered as the top of the plant. All of the tops of a single culture were cut into approximately 2-cm. lengths and placed in a weighed test-tube. When all the tops of both series had been harvested in this manner, they were weighed immediately and the weight recorded as fresh weight of tops for each culture as a whole. Thus all of the values reported in this paper respecting any particular culture refer in every case to the entire six plants in that culture, and not to a single plant.

After the fresh weight of tops had been determined the tops were dried for 24 hours at a temperature of 78°C., after which they were dried to constant weight at 102°C. The test-tubes containing the tops were transferred from the oven to the desiccator and were allowed to cool before weighing. Each tube was stoppered with a rubber stopper of known weight while being weighed, the same rubber stopper being used for all the tubes.

The root systems of each single culture were placed together and excess liquid removed by blotting paper. They were then placed in a weighed test-tube and dried to constant weight in the same manner as described for the tops.

Dry weights of entire plants

These data are obtained as the direct summation of the actual dry weights of tops and roots. The calculated relative values are shown in table 3.

The first two columns of each time-period present the data for series A (the outer row on the rotating table) and series B (the inner row on the rotating table). The data as given in the tables are not the actual weights but the relative values of the weights expressed in terms of the weight of culture R_1S_1 . The weight of culture R_1S_1 is considered as unity and its actual weight is given in parentheses, in grams. If it is desired to know the actual weight of any other culture of the series, it may be obtained by multiplying the relative weight of that culture by the actual weight of culture R_1S_1 as given in parentheses in the same column. The average seven highest cultures of each time-period are designated by H and the five cultures giving the lowest yields are designated by L.

Essentially the cultures showing the best yields of tops are the cultures which give the maximum weights of the entire plants.

To present graphically the data of table 3 use is made of triangle diagrams (fig. 2) similar to that shown in figure 1. The culture numbers are the same as those in figure 1 except that the numbers at the intersections within the triangle have been omitted as a matter of convenience. The areas with crosses on the triangle indicate the seven cultures having the highest dry weights. The areas with small circles indicate the five cultures having the lowest dry weights. The cultures having maximum and minimum weights are indicated

by large circles in their respective areas. It should be noted here that there is within the large triangle a smaller inner triangle composed of six solutions all of which offer favorable growth conditions. This triangle may be defined by giving the culture numbers at the apices, namely: R_2S_2 , R_2S_4 , R_4S_2 . The areas of low yields are confined mainly to the lower apices of the large triangle.

TABLE 3
Dry weight of entire plants

Relative dry weights of series A and B and averages, grown at the three time-periods

SOLUTION NUMBER	TIME-PERIODS								
	July 1 to August 5, 1919			November 23 to December 28, 1919			January 21 to February 25, 1920		
	A	B	Average	A	B	Average	A	B	Average
IR_1S_1	1.00L (2.10)	1.00L (1.96)	1.00L (2.03)	1.00L (0.676)	1.00L (0.658)	1.00L (0.667)	1.00L (1.53)	1.00L (1.59)	1.00L (1.56)
S_2	1.14	1.09	1.11	1.24	1.19	1.22	1.19	1.15L	1.17L
S_3	1.08	1.08L	1.08L	1.10	1.25	1.18	1.31	1.36H	1.34
S_4	1.06	1.28H	1.17	1.06	1.12L	1.09L	1.04L	1.33	1.19
S_5	1.11	1.34H	1.22	1.03	1.01L	1.02L	1.36H	1.46H	1.41H
S_6	1.03	1.10	1.06L	0.97L	0.82L	0.90L	1.29	1.29	1.29
R_2S_1	0.98L	0.97L	0.94L	0.92L	1.32	1.12	1.18L	1.10L	1.14L
S_2	1.32H	1.16	1.24H	1.30H	1.32	1.31H	1.47H	1.35	1.41H
S_3	1.55H	1.39H	1.47H	1.25H	1.32H	1.29H	1.18L	1.45H	1.32
S_4	1.42H	1.03L	1.23	1.19	1.35H	1.27H	1.34	1.46H	1.40H
S_5	1.00L	0.92L	0.96L	1.00L	1.07L	1.04L	1.45H	1.27	1.36H
R_3S_1	0.98L	1.23	1.10	1.17	1.34H	1.26	1.27	1.09L	1.18
S_2	1.24H	1.33H	1.28H	1.40H	1.26	1.33H	1.46H	1.25	1.36H
S_3	1.36H	1.29H	1.33H	1.33H	1.19	1.26	1.53H	1.54H	1.54H
S_4	1.15	1.24	1.19	1.12	1.16	1.14	1.30	1.35	1.33
R_4S_1	1.31H	1.18	1.24H	1.21	1.28	1.25	1.28	1.06L	1.17L
S_2	1.40H	1.39H	1.40H	1.19	1.32H	1.26	1.52H	1.38H	1.45H
S_3	0.98L	1.29	1.13	1.02L	1.40H	1.21	1.32	1.15	1.24
R_5S_1	1.20	1.29H	1.25H	1.47H	1.30	1.39H	1.02L	1.25	1.14L
S_2	1.16	1.26	1.20	1.25H	1.34H	1.30H	1.67H	1.44H	1.56H
R_6S_1	1.14	1.11	1.13	1.47H	1.38H	1.43H	1.28	1.29	1.29
Shive's.....			1.08*	1.40	1.33	1.42*	1.32	1.26	1.21*
K.....				1.06	1.28	1.17	1.48	1.55	1.52
T.....				1.11	1.26	1.19	1.82	1.59	1.71
Distilled H_2O .			0.16			0.05			0.23

* Average of three cultures.

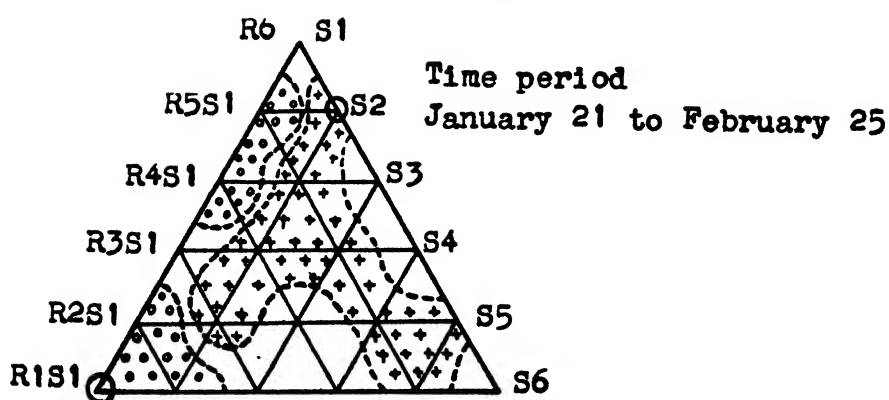
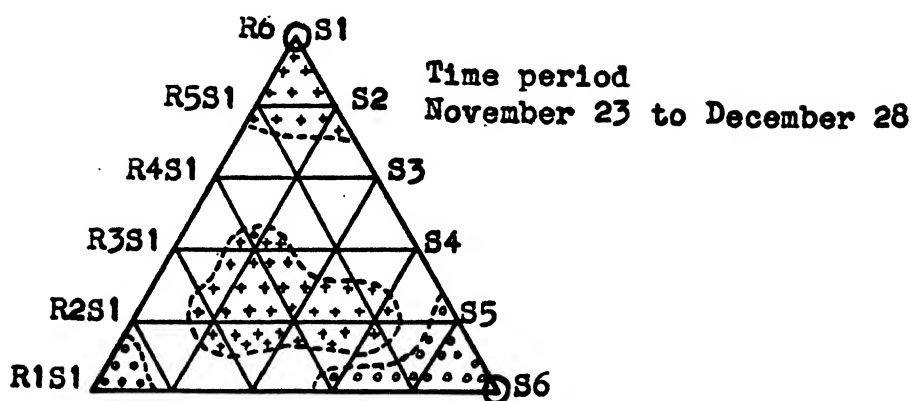
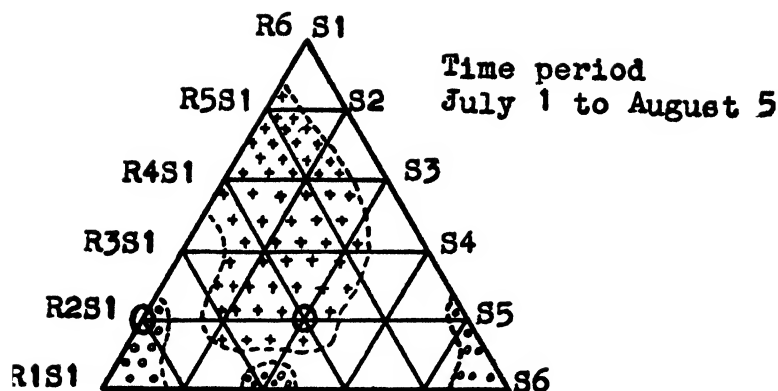


FIG. 2. DRY WEIGHT OF ENTIRE PLANTS

Diagrams showing the position of the cultures having the highest seven and lowest five relative yields, dry weight of entire wheat plants, averages of series A and B; high-yield areas are designated by crosses, areas of low yield by small circles; the cultures giving maximum and minimum yields are marked by large circles in their respective areas.

Transpiration

The total relative amounts of water (grams) absorbed by cultures are shown in table 4. These data are plotted on the triangle diagrams shown in figure 3.

TABLE 4

Transpiration

Relative amounts of transpiration of plants of series A and B, grown at three different time-periods

SOLUTION NUMBER	TIME-PERIODS								
	July 1 to August 5, 1919			November 23 to December 28, 1919			January 21 to February 25, 1920		
	A	B	Average	A	B	Average	A	B	Average
IR ₁ S ₁	1.00L (878)	1.00L (908)	1.00L (893)	1.00 (422)	1.00 (411)	1.00 (416)	1.00L (728)	1.00L (680)	1.00L (704)
S ₂	1.09L	1.06	1.07L	1.09H	0.98L	1.04	1.10	1.10	1.10
S ₃	1.07L	1.03L	1.05L	0.97L	1.04	1.00	1.14H	1.18H	1.16H
S ₄	1.24H	1.16	1.20H	0.93L	0.97L	0.95L	1.08	1.11	1.10
S ₅	1.20	1.25H	1.22H	0.98	1.00	0.99	1.15H	1.27H	1.21H
S ₆	1.09	1.03	1.06L	0.89L	0.91	0.90L	1.16H	1.18H	1.17H
R ₂ S ₁	1.01L	0.96L	0.99L	0.94L	1.04	0.99	1.03L	1.06	1.05
S ₂	1.22	1.06	1.14	1.05H	1.09H	1.07H	1.11H	1.07	1.09
S ₃	1.35H	1.15H	1.25H	1.10H	1.06H	1.08H	1.05	1.12	1.09
S ₄	1.29H	1.01L	1.15	1.05H	1.12H	1.09H	1.01L	1.07	1.04L
S ₆	1.14	1.05L	1.09	0.94L	0.92	0.93L	1.11	1.05L	1.08
R ₃ S ₁	1.14	1.18H	1.16	0.99	1.12H	1.06H	1.10	0.94L	1.02L
S ₂	1.17	1.12H	1.14	1.05	1.03	1.04	1.12H	1.13H	1.13H
S ₃	1.19	1.07	1.13	1.14H	1.02	1.08H	1.09	1.15H	1.12H
S ₄	1.17	1.13	1.15	0.96	0.92L	0.94L	1.03L	1.09	1.06
R ₄ S ₁	1.23H	1.05	1.14	1.01	1.00	1.01	1.11	1.04L	1.08
S ₂	1.28H	1.14	1.21H	0.98	0.97L	0.98L	1.12H	1.22H	1.17H
S ₃	1.04L	1.15H	1.09	1.01	1.03	1.02	1.04	1.01L	1.03L
R ₅ S ₁	1.21	1.15H	1.18H	1.18H	1.07H	1.13H	0.95L		0.95L
S ₂	1.25H	1.12H	1.18H	0.99	1.05H	1.02	1.17H	1.07	1.12H
R ₆ S ₁	1.24H	1.11	1.17H	1.14H	1.09H	1.11H	1.06	1.16H	1.11
Shive's.....			0.98*			0.97*	1.03	1.05	0.98*
K.....				0.93	0.95	0.94	1.05	1.10	1.08
T.....				0.92	0.88	0.90	1.11	1.13	1.12
Distilled H ₂ O.						0.03			0.01

* Average of three cultures.

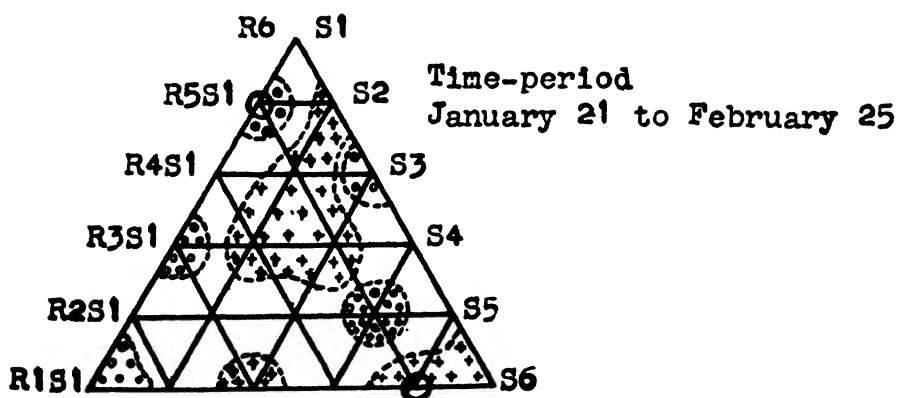
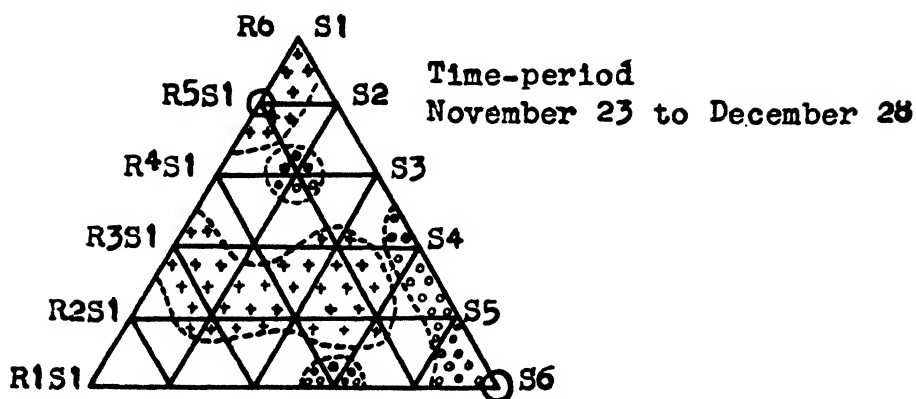
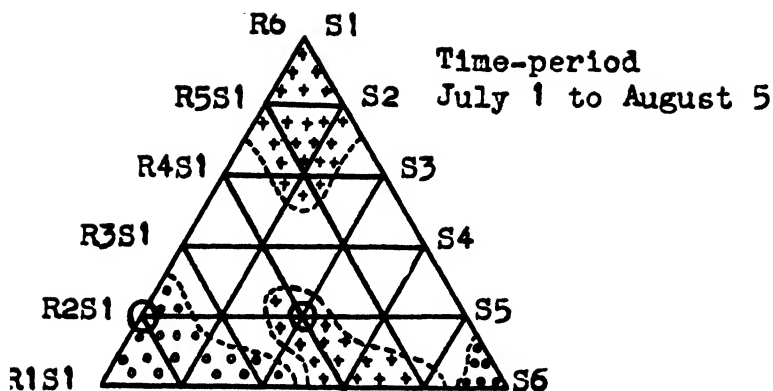


FIG. 3. TRANSPIRATION

Diagrams showing relative amounts of water lost by transpiration; areas of high transpiration indicated by crosses, areas of low transpiration indicated by small circles; the cultures showing the highest loss or lowest loss of water are designated by larger circles in their respective areas.

Water requirement

The water requirement of plants is commonly defined as the number of grams of water required to produce 1 gm. of dry weight of plant material. As calculated, it is the total number of grams of solution absorbed, divided by the dry weight of the entire plants. The data of the water-requirements are given in table 5 and figure 4.

TABLE 5

Water requirement

Showing relative amounts of water absorbed to produce 1 gm. of dry weight of plant material of series A and B and averages, conducted through three different time-periods

SOLUTION NUMBER	TIME-PERIODS								
	July 1 to August 5, 1919			November 23 to December 28, 1919			January 21 to February 25, 1920		
	A	B	Average	A	B	Average	A	B	Average
IR ₁ S ₁	1.00 (414)	1.00H (463)	1.00 (438)	1.00H (625)	1.00H (626)	1.00H (625)	1.00H (476)	1.00H (427)	1.00H (451)
S ₂	0.97	0.97	0.97	0.87	0.82	0.85	0.93H	0.96H	0.95H
S ₃	0.99	0.96	0.98	0.89	0.83	0.86	0.87	0.86	0.87
S ₄	1.18H	0.90	1.04H	0.88	0.86	0.87	1.03H	0.84	0.94
S ₅	1.10	0.93	1.02H	0.96H	0.98H	0.97H	0.84	0.87	0.86
S ₆	1.07	0.93	1.00	0.92	1.11H	1.02H	0.90H	0.92H	0.91H
R ₂ S ₁	1.13H	0.99H	1.06H	0.99H	0.79L	0.89	0.87	0.96H	0.92H
S ₂	0.94L	0.92	0.93L	0.81L	0.82	0.82L	0.75L	0.79L	0.77L
S ₃	0.88L	0.83L	0.86L	0.88	0.80L	0.84	0.89	0.77L	0.83
S ₄	0.90L	0.98H	0.94L	0.88	0.83	0.86	0.76L	0.73L	0.75L
S ₅	1.15H	1.14H	1.15H	0.94H	0.87H	0.91H	0.76L	0.83L	0.80L
R ₃ S ₁	1.17H	0.96	1.07H	0.85	0.82	0.84	0.86	0.92	0.89
S ₂	0.95L	0.84L	0.90L	0.75L	0.81	0.78L	0.77L	0.91	0.84
S ₃	0.88L	0.82L	0.85L	0.86	0.85	0.86	0.71L	0.75L	0.73L
S ₄	1.03	0.91	0.97	0.86	0.80L	0.83L	0.79	0.81L	0.80L
R ₄ S ₁	0.95L	0.89L	0.92L	0.84L	1.10H	0.97H	0.86H	0.96H	0.91H
S ₂	0.92L	0.82L	0.87L	0.82L	0.73L	0.78L	0.74L	0.88	0.81L
S ₃	1.07	0.90	0.99	0.99H	0.74L	0.87	0.79	0.88	0.84
R ₅ S ₁	1.02	0.89L	0.96	0.81L	0.83	0.82L	0.93H	†	†
S ₂	1.09	0.89L	0.99	0.79L	0.79L	0.79L	0.70L	0.74L	0.72L
R ₆ S ₁	1.10H	0.99H	1.05H	0.78L	0.78L	0.78L	0.83	0.90	0.87
Shive's.....	0.97	0.91	0.94*	0.75	0.71	0.69*	0.78	0.83	0.82*
K.....				0.88	0.75	0.82	0.71	0.72	0.72
T.....				0.83	0.70	0.77	0.61	0.71	0.66
Distilled H ₂ O.			0.63			0.67			0.74

* Average of three cultures.

† Container overturned.

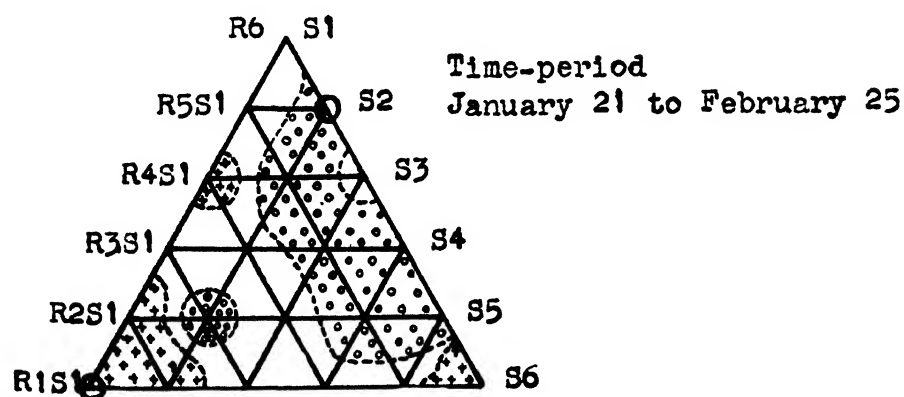
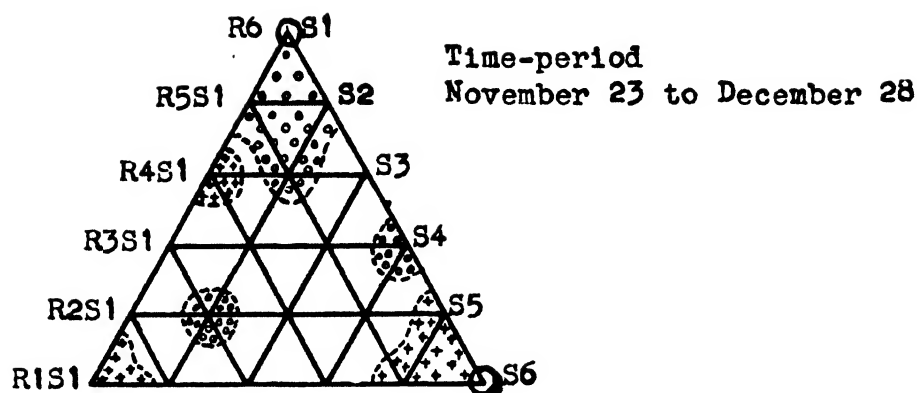
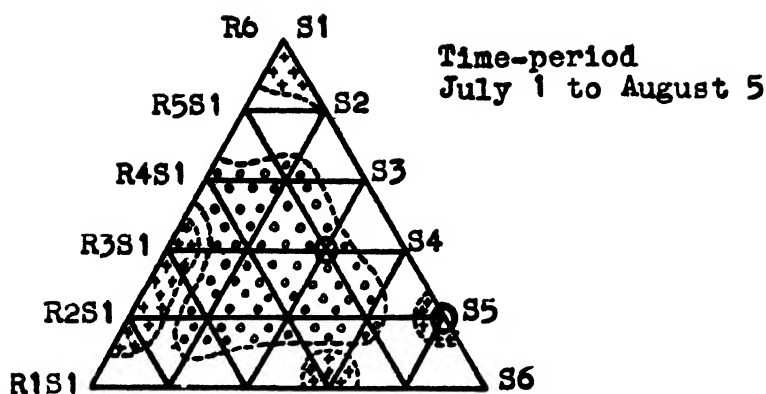


FIG. 4. WATER REQUIREMENTS

Diagrams showing the position of the cultures having the highest five and lowest seven relative water-requirements, averages of series A and B, grown at three different time-periods; areas of high water-requirements are marked by crosses, areas of low water-requirements by small circles; the cultures having the maximum and minimum water-requirements are designated by large circles in their respective areas.

Hydrogen-ion concentration

Measurements of the hydrogen-ion concentration were made of the nutrient solutions at the beginning of each time-period and again at the end of each $3\frac{1}{2}$ -day interval. The data thus obtained are presented in tables 6, 7 and 8.

TABLE 6

Showing changes of pH at the end of each $3\frac{1}{2}$ -day interval, averages of series A and B, conducted from July 1 to August 5, 1919, inclusive

SOLUTION NUMBER	JULY									AUGUST	
	1	4	8	11	15	18	22	25	29	1	5
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
IR ₁ S ₁	5.3	5.6	6.4	6.4	7.0	6.8	7.0	6.8	6.9	6.9	6.8
S ₂	5.3	5.8	6.5	6.4	7.2	6.9	7.0	6.9	7.1	6.9	6.6
S ₃	5.2	5.8	6.4	6.4	6.9	6.8	7.1	6.8	6.9	6.9	7.1
S ₄	5.2	5.9	6.5	6.4	6.8	7.1	7.1	6.6	6.2	6.2	5.7
S ₅	5.2	5.9	6.3	6.3	6.7	6.4	6.2	6.1	5.7	6.2	5.6
S ₆	5.2	5.9	6.1	6.1	6.5	6.2	6.1	5.8	5.8	6.0	5.6
R ₂ S ₁	5.0	5.4	6.1	6.1	6.5	6.5	6.3	6.3	6.2	6.3	6.1
S ₂	4.9	5.5	6.2	6.2	6.4	6.3	6.2	6.1	6.1	6.6	5.8
S ₃	4.9	5.5	6.1	6.1	6.2	6.1	5.8	5.7	5.5	6.2	5.1
S ₄	4.9	5.4	6.1	6.0	6.0	5.9	5.6	5.6	5.4	5.8	4.9
S ₅	4.9	5.6	5.9	5.9	5.8	5.8	5.4	5.5	5.3	5.5	5.2
R ₃ S ₁	4.9	5.3	6.1	6.1	6.2	6.3	6.1	6.1	5.6	6.4	5.7
S ₂	4.9	5.5	6.1	6.1	6.1	6.0	5.7	5.7	5.5	5.8	5.3
S ₃	4.9	5.3	5.9	5.9	5.8	5.5	5.4	5.5	5.3	5.6	5.0
S ₄	4.9	5.4	5.7	5.7	5.6	5.4	5.3	5.2	5.0	5.3	4.9
R ₄ S ₁	4.9	5.3	5.9	6.0	6.1	6.1	5.8	5.9	5.9	6.2	5.7
S ₂	4.9	5.2	5.9	5.9	5.9	5.8	5.4	5.4	5.3	5.5	5.0
S ₃	4.9	5.3	5.7	5.5	5.5	5.3	5.2	5.1	5.0	5.3	4.9
R ₅ S ₁	4.7	5.3	5.9	5.9	5.9	6.0	5.7	5.7	5.5	5.9	5.4
S ₂	4.7	5.2	5.7	5.6	5.5	5.5	5.2	5.2	5.0	5.4	4.7
R ₆ S ₁	4.9	5.2	5.9	5.9	5.8	5.9	5.6	5.5	5.4	5.6	5.2
Shive's*.....	4.6†	4.9	5.4	5.5	5.5	5.7	5.5	5.5	5.4	5.5	5.3
K.....											
T.....											
Distilled H ₂ O.....	5.8	6.4	7.0	6.3	6.3	6.7	6.3	6.3	7.8	7.1	6.2

* Averages of 3 cultures.

† Shive (15) reports the value, pH = 4.7; the difference between our values has little significance, since it is within the limit of experimental error for the colorimetric method

It will be observed that the difference between the initial and final pH (fig. 5) for a given $3\frac{1}{2}$ -day interval is greater when the nutrient solution contains only small amounts of the phosphate radical. Thus, the solutions along the basal

line of the triangle show the greatest changes in pH due, apparently, to the fact that they are insufficiently buffered by the lesser quantities of KH_2PO_4 . While the solutions near or at the upper apex of the triangle are more highly

TABLE 7

Showing changes of pH at the end of each 3½-day interval, averages of series A and B, conducted from November 23 to December 28, 1919, inclusive

SOLUTION NUMBER	NOVEMBER			DECEMBER							
	23	26	30	3	7	10	14	17	21	24	28
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
IR ₁ S ₁	5.3	5.5	5.6	6.1	6.5	6.0	6.3	6.1	5.8	5.4	5.4
S ₂	5.3	5.4	5.6	6.1	6.7	6.1	6.4	6.1	6.1	6.0	5.5
S ₃	5.2	5.4	5.5	6.1	6.6	6.2	6.5	6.2	6.1	5.4	5.2
S ₄	5.2	5.5	5.6	6.1	6.7	6.1	6.5	6.1	6.0	5.8	5.4
S ₅	5.2	5.6	5.6	6.2	6.6	6.2	6.4	6.1	6.0	5.6	5.6
S ₆	5.2	5.5	5.7	6.3	6.3	6.1	6.1	6.0	6.0	5.9	5.7
R ₂ S ₁	5.0	5.2	5.4	5.7	6.1	5.7	6.0	5.7	5.6	5.4	5.0
S ₂	4.9	5.3	5.3	5.8	6.3	5.9	6.1	6.0	6.0	5.8	5.3
S ₃	4.9	5.3	5.4	5.9	6.3	5.9	6.1	6.0	6.0	5.7	5.3
S ₄	4.9	5.3	5.4	5.9	6.3	5.9	6.1	6.0	5.9	5.7	5.4
S ₅	4.9	5.3	5.4	5.9	6.2	6.0	5.9	5.9	5.8	5.6	5.5
R ₃ S ₁	4.9	5.1	5.3	5.8	6.1	5.6	5.9	5.8	5.7	5.5	5.1
S ₂	4.9	5.1	5.3	5.5	6.1	5.6	6.0	5.9	5.8	5.7	5.1
S ₃	4.9	5.2	5.3	5.7	6.1	5.8	6.0	5.9	5.7	5.5	5.0
S ₄	4.9	5.2	5.3	5.6	6.1	5.7	5.7	5.7	5.6	5.5	5.4
R ₄ S ₁	4.9	5.0	5.2	5.5	5.9	5.5	5.9	5.9	5.6	5.5	4.9
S ₂	4.9	5.0	5.2	5.5	5.9	5.6	5.9	5.6	5.6	5.5	4.9
S ₃	4.9	5.0	5.2	5.5	5.9	5.6	5.7	5.7	5.5	5.5	5.3
R ₅ S ₁	4.7	4.9	5.1	5.5	5.9	5.5	5.8	5.7	5.6	5.5	4.8
S ₂	4.7	4.9	5.1	5.5	6.0	5.6	5.9	5.6	5.5	5.4	4.9
R ₆ S ₁	4.9	4.8	5.1	5.5	5.9	5.5	5.8	5.6	5.6	5.5	4.9
Shive's*.....	4.6†	4.6	4.7	5.2	5.5	5.2	5.5	5.4	5.3	5.4	4.7
K.....	5.1	5.1	5.3	5.9	6.1	5.8	5.9	6.3	6.2	5.5	5.4
T.....	4.9	4.6	5.1	5.4	5.8	5.4	5.7	6.1	5.9	5.1	4.8
Distilled H ₂ O.....	5.8	6.3	5.6	6.3	5.9	6.5	6.3	6.3	5.7	7.4	6.2

* Averages of 3 cultures.

† Shive (15) reports the value, pH = 4.7; the difference between our values has little significance, since it is within the limit of the experimental error for the colorimetric method.

buffered because of the increased quantity of the phosphate salt present, they are also more highly acid (the pH is less), because of the dissociation of the hydrogen-ion in this salt. These observations are in harmony with Salter

and McIlvaine. The reaction of all the solutions in which plants had been grown was toward the neutral point ($\text{pH} = 7$).²

TABLE 8

Showing changes of pH at the end of each 3½-day interval, averages of series A and B, conducted from January 21 to February 25, 1920, inclusive

SOLUTION NUMBER	JANUARY			FEBRUARY							
	21	25	28	1	4	8	11	15	18	22	25
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
IR ₁ S ₁	5.3	5.6	6.2	6.6	6.7	6.5	6.7	7.0	6.7	6.9	6.4
S ₂	5.3	5.7	6.2	6.6	6.8	6.6	6.7	7.1	6.9	7.1	6.8
S ₃	5.2	5.4	5.9	6.2	6.4	6.2	6.3	7.3	6.9	7.5	7.2
S ₄	5.2	5.6	6.1	6.4	6.7	6.6	6.7	7.1	6.7	7.4	6.8
S ₅	5.2	5.8	6.3	6.6	6.5	6.2	6.3	6.6	6.3	7.2	6.8
S ₆	5.2	5.7	6.3	6.5	6.3	6.1	6.4	6.6	6.4	7.4	6.5
R ₄ S ₁	5.0	5.3	5.9	6.2	6.4	6.3	6.5	6.6	6.4	6.7	6.7
S ₂	4.9	5.3	5.9	6.3	6.5	6.3	6.4	6.7	6.5	7.0	6.3
S ₃	4.9	5.4	5.9	6.2	6.4	6.2	6.3	6.5	6.3	6.4	6.3
S ₄	4.9	5.4	5.9	6.1	6.2	6.0	6.1	6.3	6.1	6.3	6.2
S ₅	4.9	5.4	5.9	6.1	6.0	5.6	5.9	6.0	5.9	5.8	6.0
R ₃ S ₁	4.9	5.1	5.7	6.0	6.1	6.0	6.3	6.5	6.4	6.2	6.2
S ₂	4.9	5.3	5.8	6.0	6.2	6.1	6.3	6.4	6.2	6.1	6.2
S ₃	4.9	5.3	5.7	6.0	6.1	5.8	6.0	6.1	5.9	5.8	5.7
S ₄	4.9	5.2	5.6	5.9	5.9	5.4	5.6	5.6	5.4	5.3	5.3
R ₄ S ₁	4.9	5.1	5.6	5.9	6.1	5.9	6.2	6.4	6.3	6.0	6.0
S ₂	4.9	5.1	5.6	6.0	6.1	5.9	6.0	6.1	5.9	5.5	5.6
S ₃	4.9	5.2	5.6	5.8	5.8	5.3	5.4	5.4	5.5	5.0	5.3
R ₅ S ₁	4.7	5.0	5.4	5.8	6.0	5.8	6.1	6.2	6.1	5.8	5.7
S ₂	4.7	5.1	5.6	5.8	5.8	5.5	5.6	5.5	5.5	5.3	5.5
R ₄ S ₁	4.9	4.9	5.4	5.9	6.0	5.7	6.1	6.0	5.9	5.6	6.0
Shive's*.....	4.6†	4.8	5.1	5.4	5.6	5.4	5.6	5.6	5.5	5.5	5.5
K.....	5.1	5.4	5.9	6.1	6.0	5.8	6.1	6.3	6.1	6.0	5.9
T.....	4.9	5.0	5.4	5.8	5.7	5.5	5.5	5.6	5.4	5.1	5.3
Distilled H ₂ O.....	5.8	6.6	6.3	6.1	5.9	4.7	5.0	5.1	5.0	5.6	5.5

* Averages of 3 cultures.

† Shive (15) reports the value $\text{pH} = 4.7$; the difference between our values has little significance, since it is within the limit of the experimental error for the colorimetric method.

² This in general substantiates the observations of Itano (6), Hoagland (5), Salter and McIlvaine (12), and Duggar (2).

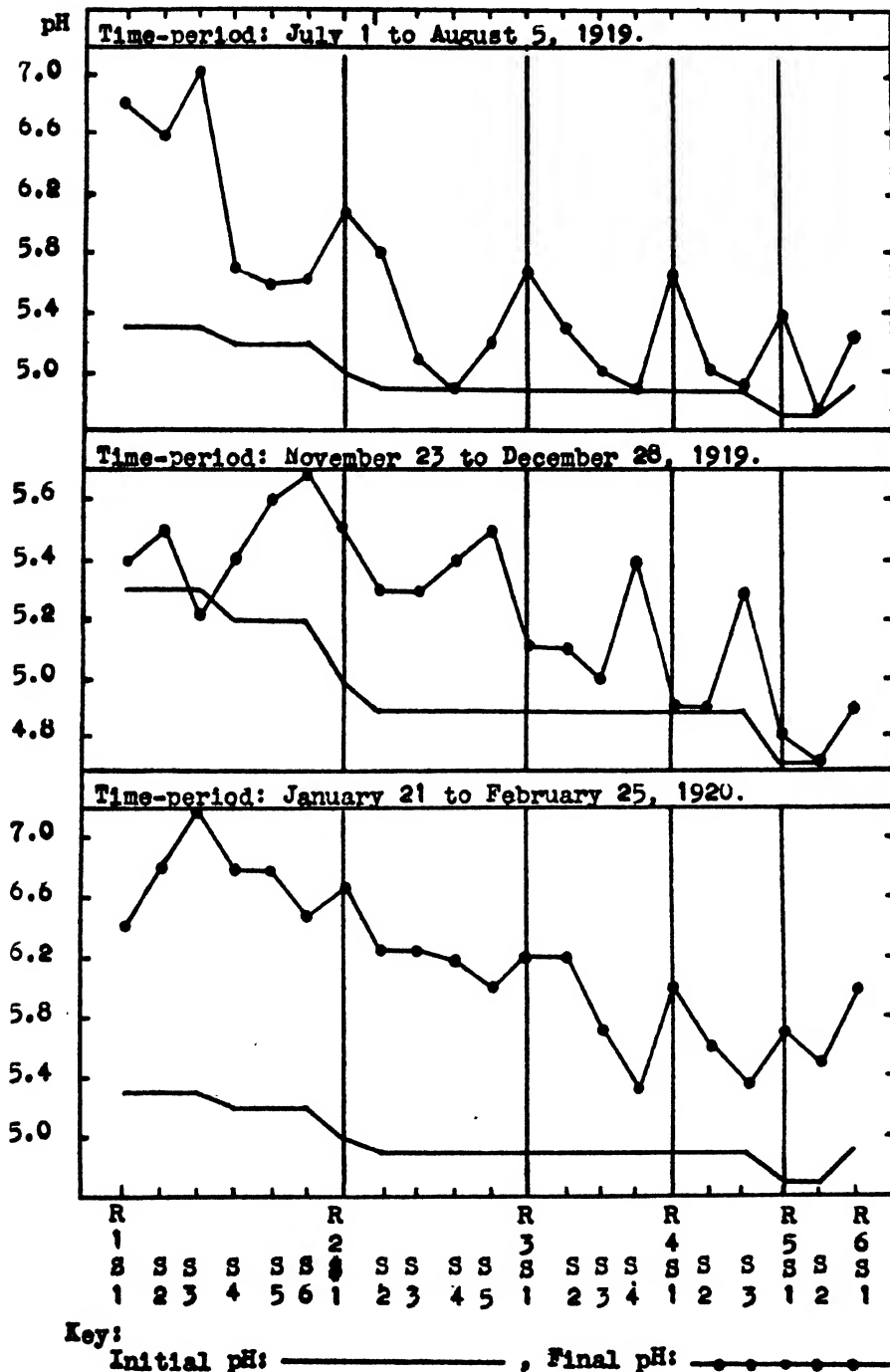


FIG. 5. DIAGRAMS SHOWING THE RELATION BETWEEN THE INITIAL pH VALUES, AND FINAL pH VALUES, AVERAGES OF SERIES A AND B, FOR THE THREE TIME-PERIODS

Condition of plants

The roots of the cultures throughout the three time-periods were fairly uniform. Roots of R_1S_1 , R_1S_2 , R_1S_3 were shorter, often of a brownish color, and had fewer secondary rootlets.

No very significant differences in size or condition of tops were noticeable until near the end of the growth period. At that time there were noticeable differences in height as well as apparent differences in vigor of plants; these differences were closely connected with the dry weights of cultures. The growth of the cultures during the second time-period was poor, due first of all to poor seasonal light conditions and second, to mildew infection.

Cultures of all three time-periods were infected to a greater or less extent with mildew but this infection never became a limiting factor except possibly in the cultures of the second time-period.

So-called magnesium or tip injury appeared in cultures R_1S_1 , R_2S_1 , R_3S_1 , R_4S_1 , R_5S_1 , and R_6S_1 . The greatest amount of this injury occurred in culture R_1S_1 and the injury declined progressively toward the upper apex.

It may be that the yellowing of the leaves of potato plants grown in sand-cultures as reported by Johnston (7) is due in part at least to an excess of magnesium, for his data show that the plants having the greatest approximate percentage of yellow leaf-area are the plants grown in solutions R_1S_1 , R_2S_1 , R_3S_1 , etc. However, he makes no mention of the correlation.

DISCUSSION

Comparison of various weight data

The cultures of the first time-period having maximum and minimum yields, R_2S_3 and R_2S_1 , respectively, show the same relative transpiring powers. During the second time-period there is agreement between the cultures showing minimum transpiration; though the culture having the highest transpiring power, R_5S_1 , does not have the maximum weight. There is good general agreement between transpiration quantities and yield of plants during the third time-period. As an indicator of relative physiological activity, including growth, transpiration quantities are valuable; but their value rests chiefly in the corroborative evidence which they offer in support of other data. Our data as to the general relation between growth and transpiration support Shive's (14) conclusion that "water transpired appears to be as good a criterion as is the final dry weight, for judging the comparative growth obtained in the different solutions." The authors, however, have not found any direct correlation between high root-yields and low transpiration as he suggests.

Water-requirement and dry weight

It was noticed at the time the water-requirement data were calculated, that the cultures having the lowest water-requirements were the cultures that gave the total maximum yields of plants. A comparison of the triangles of the

three time-periods will show how consistently this relation held true. The areas showing high yields of plants correspond with the areas showing low water-requirements. The relation between transpiration and water-requirements is in general the same as for yield of plants and water-requirements; cultures having a high water-requirement have low transpiring powers and the minimum dry-weight yields.

Relation of ion ratios to yields

The high-yield areas (fig. 2) tend to concentrate themselves in the central portion of the triangle diagrams, whereas the maximum and minimum ion ratio values are grouped along the marginal lines of the triangles, leaving an unoccupied space in the center. Reference to table 9 giving the actual values

TABLE 9

Cation ratios—Values of the three cation ratios and the pH values of the nutrient solutions used in the experiments reported herein

SOLUTION NUMBER	Mg/Ca	Mg/K	Ca/K	pH*
IR ₁ S ₁	5.96H	5.96H	1.00	5.3
S ₂	2.51H	4.92H	1.96H	5.3
S ₃	1.32	3.91H	2.95H	5.2
S ₄	0.75	3.04H	4.04H	5.2
S ₅	0.39L	1.95H	4.90H	5.2
S ₆	0.16L	1.00H	6.10H	5.2
R ₁ S ₁	4.88H	2.49H	0.51	5.0
S ₂	2.02H	2.02H	1.00	4.9
S ₃	1.00	1.51	1.51	4.9
S ₄	0.50	1.00	2.00H	4.9
S ₅	0.20L	0.51	2.53H	4.9
R ₄ S ₁	4.04H	1.32	0.32L	4.9
S ₂	1.50	1.00	0.66	4.9
S ₃	0.66	0.66	1.00	4.9
S ₄	0.24L	0.32L	1.32	4.9
R ₄ S ₁	2.96H	0.74	0.25L	4.9
S ₂	1.00	0.50	0.50	4.9
S ₃	0.32L	0.24L	0.75	4.9
R ₆ S ₁	2.04H	0.39L	0.19L	4.7
S ₂	0.48	0.19L	0.39L	4.7
R ₆ S ₁	1.00	0.16L	0.16L	4.9
Shive's.....	2.88	0.83	0.29	4.6

* Note: After this article had gone to press, McCall and Hagg reported (Soil Science, v, 10, p. 481-485) the pH values of all 6 types of solutions. It should be noted that their values for type I vary from ours by an approximate difference of 0.5 pH.

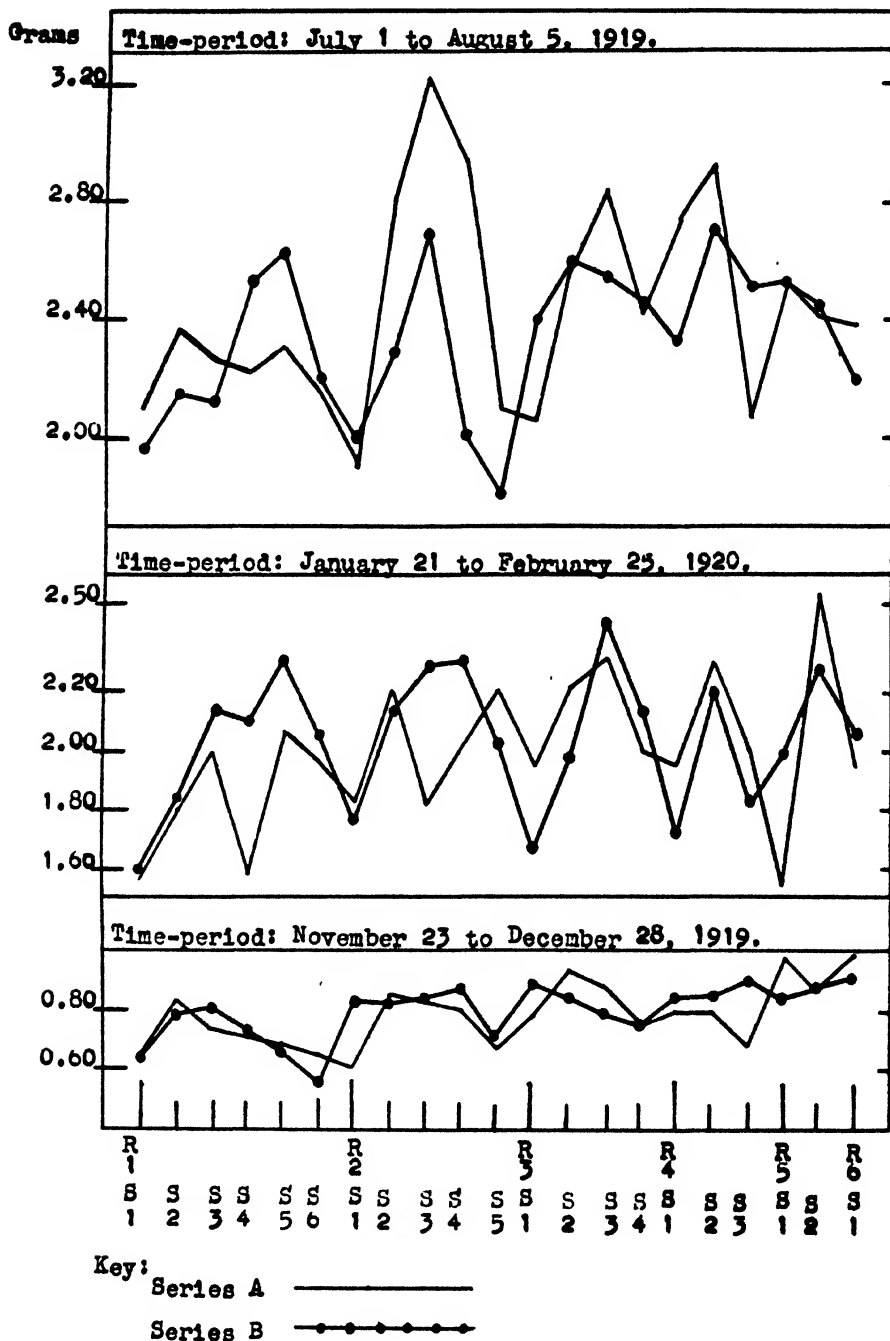
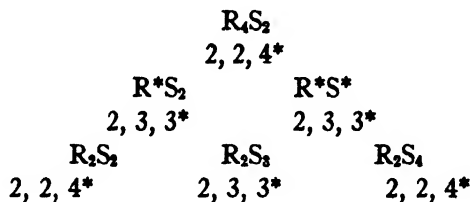


FIG. 6. DIAGRAMS TO SHOW THE RELATION OF THE DRY WEIGHT OF THE ENTIRE PLANTS OF SERIES A AND B, TO THEIR POSITION ON THE TRIANGLE DIAGRAM, AND THE CLOSE AGREEMENT BETWEEN THE TWO SERIES, A AND B, DURING EACH TIME-PERIOD

of these ratios will show that the solutions in this unoccupied central portion of the triangles have ratios of intermediate value with a tendency toward unity. Attention has been called to the fact that there seems to be an inner triangle, all the cultures of which, by reason of their salt balance, seem to offer optimum growth conditions. This inner triangle has as its apices cultures R_2S_2 , R_4S_2 , and R_2S_4 . These solutions are all characterized by ion ratios which tend to become unity in respect to each other. The molecular proportions of the three salts of this inner triangle show this same relation thus:



Hydrogen-ion concentration and plant yields

A comparison of the hydrogen-ion concentrations of the solutions (fig. 5 and 6) giving high and low yields shows that the pH does not at least become a limiting factor, in spite of the rather high initial acidity of the solutions in the upper portion of the triangle. Solutions which are poorly buffered because of the small quantity of phosphate present and hence show maximum changes of pH during the $3\frac{1}{2}$ -day interval, are the solutions which support minimum-weight yields. It is doubtful in this instance that it is the absence of sufficient buffer material that results in poor-weight yields; rather it may be the absence of sufficient proper nutrient salts.

Hydrogen-ion concentration—transpiration

Figures 7, 8 and 9 show the amounts of solution absorbed during each $3\frac{1}{2}$ -day interval for each time-period. The number of grams of solution absorbed are plotted as ordinates, and the days on which the solutions were renewed, as abscissae. The pH of the solutions at the end of each $3\frac{1}{2}$ -day interval is plotted on the same diagrams, with the pH values as ordinates. Cultures R_1S_1 , R_3S_3 and R_4S_1 are thus plotted.

The alteration in the reaction of the culture solutions in which the wheat plants had been grown increases with the age of the plants, for the first few weeks of growth. The alteration is due probably to either one of two things, the excessive withdrawal of certain ions from the solution, or the excretion of ions by the plants. The fact that for a time the increase in alkalinity of

* These figures refer to the molecular proportions of the three component salts (table 1). but for the sake of emphasis, the order of the arrangement of the components has been varied in some instances.

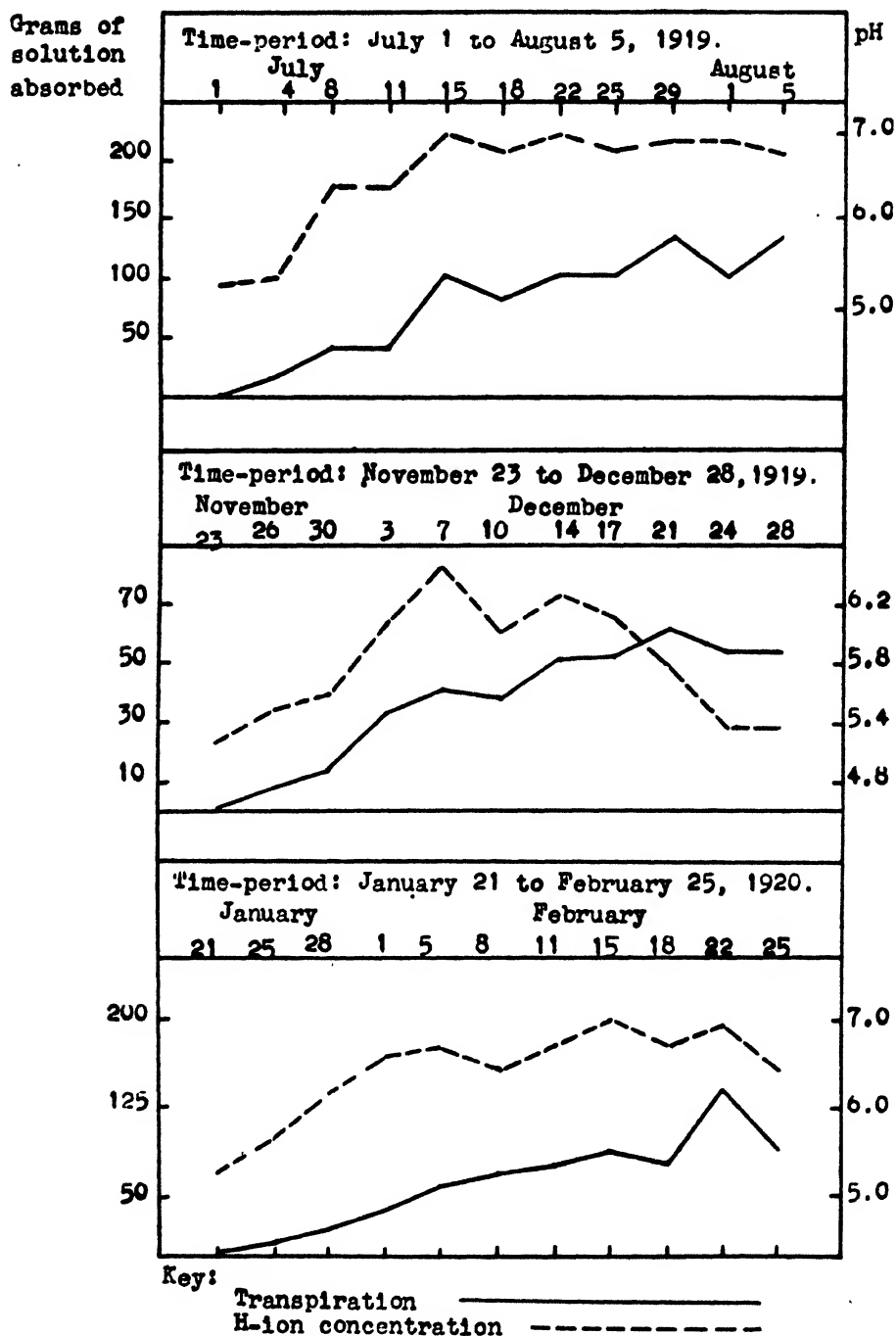


FIG. 7. DIAGRAMS SHOWING THE RELATION BETWEEN THE CHANGE OF pH AND THE GRAMS OF SOLUTION ABSORBED BY R_1S_1 , AVERAGES OF SERIES A AND B, AS MEASURED AT THE END OF EACH 34-DAY INTERVAL

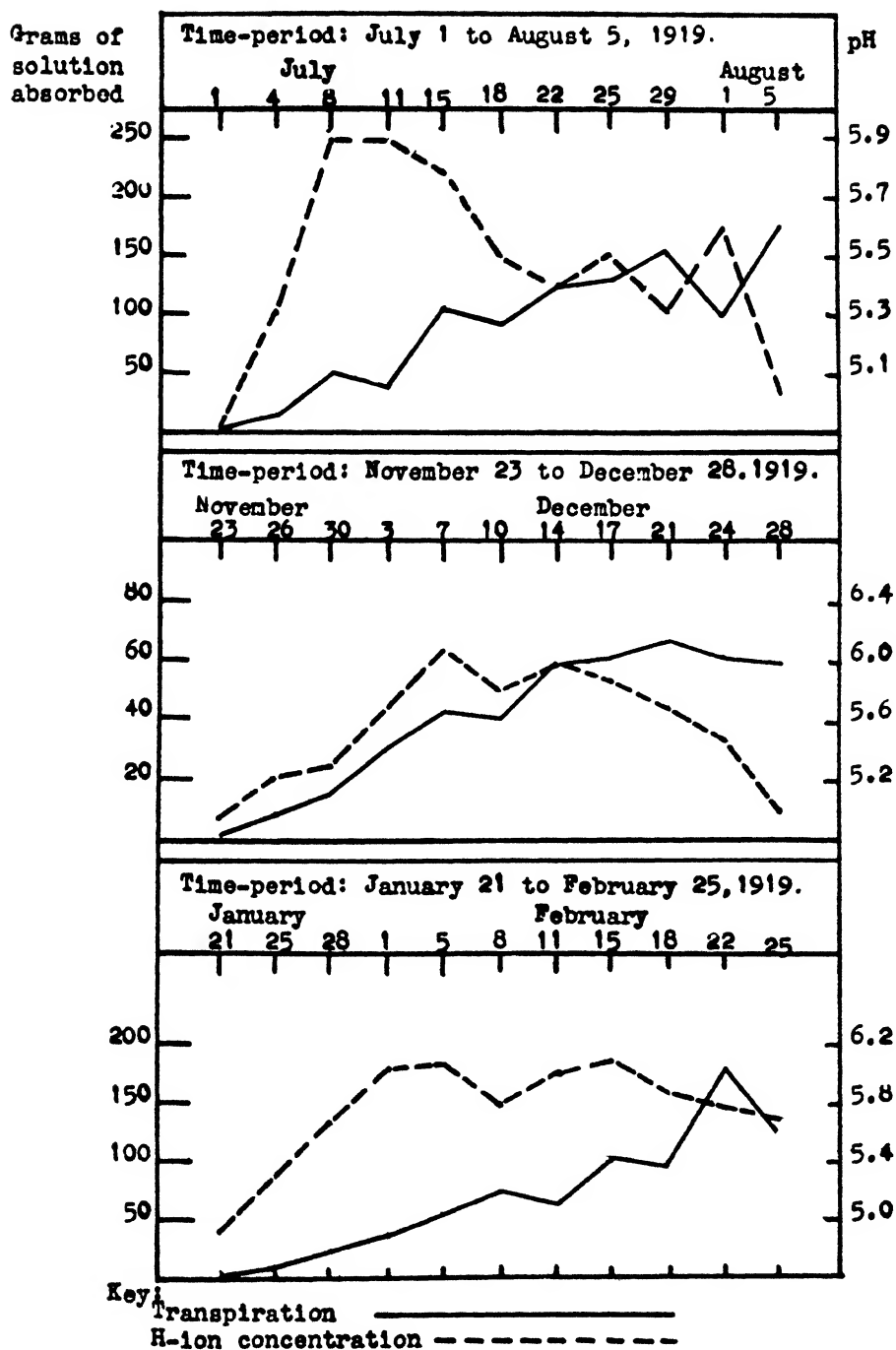


FIG. 8. DIAGRAM SHOWING THE RELATION BETWEEN THE CHANGE OF pH AND THE NUMBER OF GRAMS OF SOLUTION ABSORBED BY R_4S_4 , AVERAGES OF SERIES A AND B, AS MEASURED AT THE END OF EACH $3\frac{1}{2}$ -DAY INTERVAL

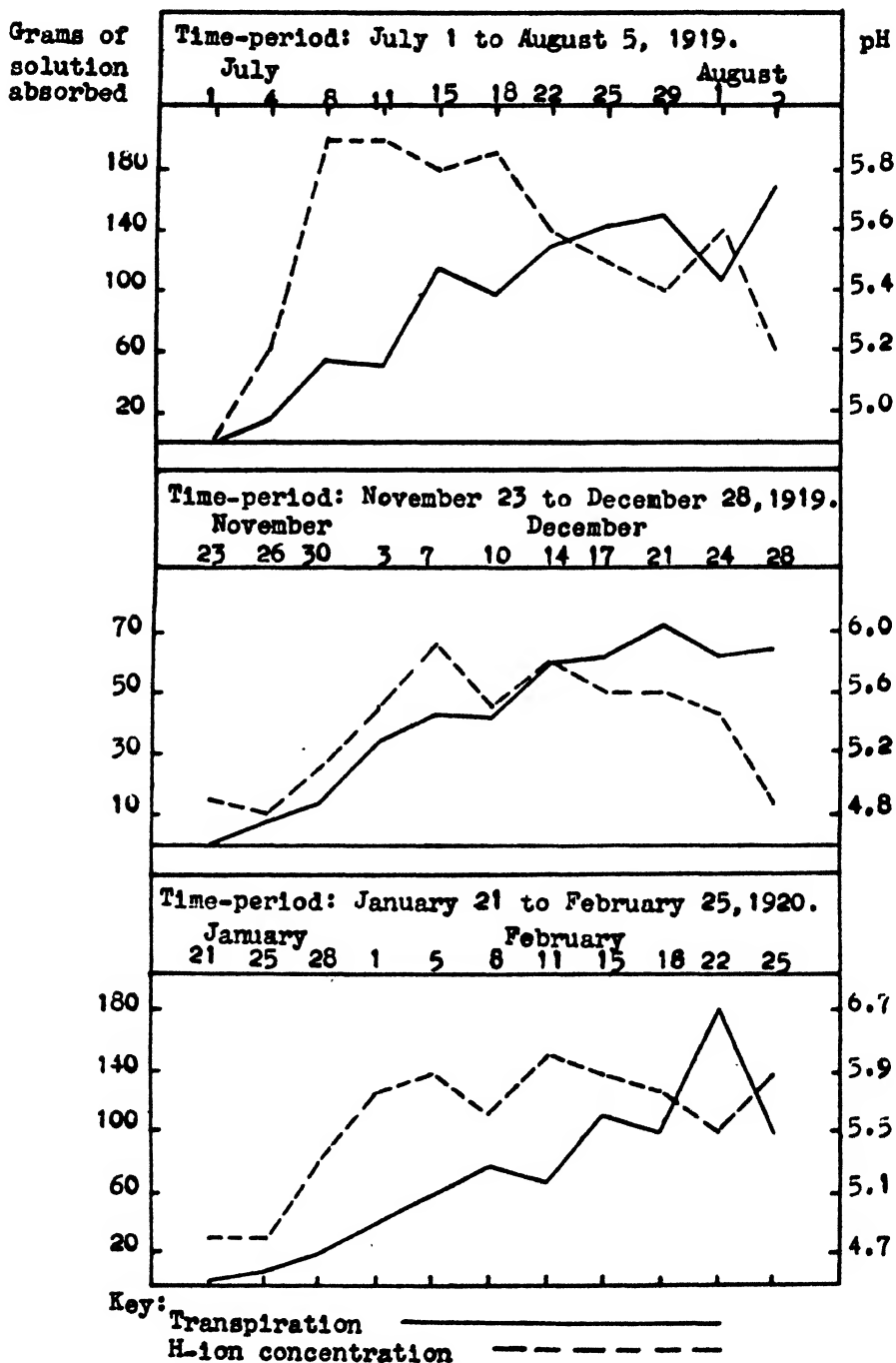


FIG. 9. DIAGRAM SHOWING THE RELATION BETWEEN THE CHANGE OF pH AND THE NUMBER OF GRAMS OF SOLUTION ABSORBED BY R_4Si , AVERAGES OF SERIES A AND B, AS MEASURED AT THE END OF EACH 3½-DAY INTERVAL

the solutions tends to parallel the course of absorption would suggest that the change of pH may be due in part to the absorption of certain ions from the solutions.

The figures indicate that the ionic absorption, if such it is, apparently takes place in relatively the same manner in all of the solutions. A quantitative analysis of the solutions would in a large measure indicate whether or not the change in reaction was due in any way to the absorption of certain of the ions.

SUMMARY

1. Wheat plants were grown 5 weeks in duplicate series, during three different time-periods, in water-culture solutions composed of the three main salts varied in increments of $\frac{1}{8}$, all having an osmotic value of 1 atmosphere. The salts used were: KH_2PO_4 , MgSO_4 and $\text{Ca}(\text{NO}_3)_2$, together with a "trace" of FePO_4 .

2. Record was taken of the aerial conditions such as temperature, relative humidity, evaporating power of the air, effective radio-intensity, and daily hours of sunshine. The cultures grown during the first time-period, July 1 to August 5, were conducted under the most favorable environmental conditions, as stated in terms of total hours of sunshine and effective radio-intensity. The environmental conditions of the cultures grown during the third time-period, January 21 to February 25, were less favorable, when judged by the same criteria. The conditions under which the cultures of the second time-period, November 23 to December 28, were grown, were least favorable toward promoting maximum growth. All the plant-measurement data were shown to correspond to these seasonal variations.

3. No culture gave consistently maximum yields of tops, roots, or total dry weight throughout the three time-periods. Cultures R_2S_2 , R_2S_3 , R_2S_4 , R_3S_2 , R_3S_4 , and R_4S_2 were usually included among the seven maximum cultures of each period. There was good agreement, however, between duplicate series of a given time-period.

4. Comparison of the water-requirements and the dry-weight yields show that the cultures having the maximum dry weights have the minimum water-requirements.

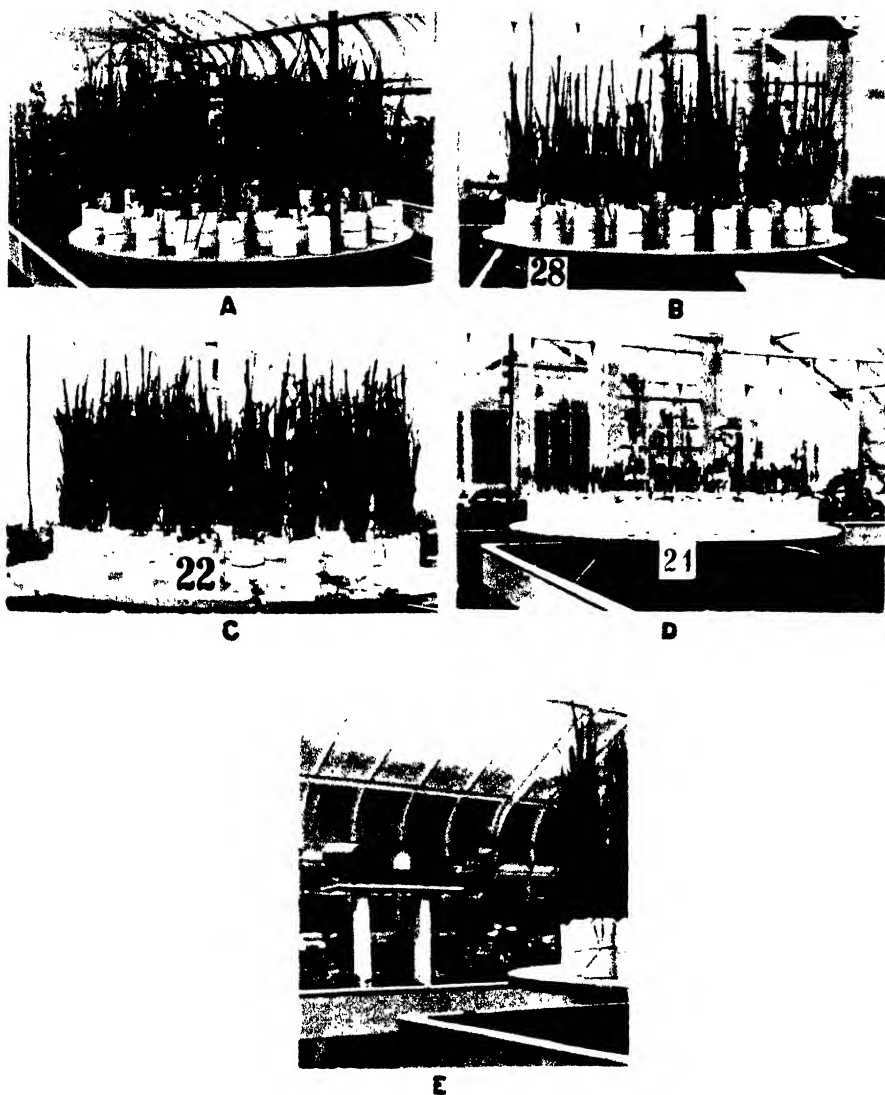
5. Data are presented which show that the hydrogen-ion concentration of solutions in which wheat has been grown tends to become less than the initial reaction of the solution. Thus all cultures were acid at the beginning and tended to become neutral. No data were obtained which explain the changes of pH in the solution. It was suggested that these differences may have been due to the selective absorption by the plant of certain ions from the solution.

6. There was no apparent direct correlation between the yield of the plants and the pH or the change of pH. Growth was generally less in those cultures (R_1S_1 and R_1S_6) which were poorly buffered, because of the insufficient quantity

of monopotassium phosphate present. They do show that degrees of acidity which have proven inhibitive to such microorganisms as *Actinomyces* and *Azotobacter* have no observable effect upon the growth of the wheat plant.

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A. Cultures of series A and B on rotating table at the end of the first time-period, July 1 to August 5, 1919.

B. Cultures of series A and B on rotating table at the end of the second time-period, November 23 to December 28, 1919.

C. Cultures of series A and B on rotating table at the end of the third time period, January 21 to February 25, 1920.

D. Cultures of series A and B on rotating table at the beginning of the second time-period, November 24, 1919. The black and white porous cup atmometers may be seen just above the tops of the cultures.

E. Showing the relative position of the thermohygrograph, on top of the two white supports, to the cultures on the rotating table, at the right.

ACID SOIL STUDIES: I. A STUDY OF THE BASIC EXCHANGE BETWEEN SOIL SEPARATES AND SALT SOLUTIONS

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INTRODUCTION

Most soils of Western Oregon, comprising chiefly the Willamette Valley and coast-lands are acid according to the usual methods now employed for the estimation of soil acidity. Field experiments show that while most of these acid soils respond to lime treatment, apparently some of them do not, as is indicated by the failure to get an increase in crop yields from the application of lime. Furthermore, some soils that may be judged neutral or only slightly acid respond well to lime treatment. A study of these different acid soils was undertaken with the hope that some differential factors might be disclosed that would explain the reason why some acid soils respond to lime treatment and others do not.

A phase of the work reported herein considers the reactions of several salt solutions on the soil separates that were segregated by a mechanical analysis of the samples. A review of the literature does not reveal any investigational work that has been done on the soil separates that would give light on the nature of the acidity, whether the sand fraction would show a greater or less acidity than the clay fraction or other points that might bear on the subject at hand.

PROCEDURE

Four characteristic acid soils were selected for the study and given the laboratory numbers 11076 to 11080, inclusive. The lime requirements determined by two methods and the classification according to the nomenclature of the Bureau of Soils, U. S. Department of Agriculture, are given in table 1.

Observations of field tests subsequently confirmed by pot experiments showed that applications of lime in different amounts to soil 11077 did not increase either legumes or grain crops.

The soil separates were obtained by a mechanical analysis of the different samples and each subdivision was isolated for study. In all samples there was only a very small amount of fine gravel which was subsequently discarded. The coarse and medium sand were combined and listed as coarse sand. The fine sand and the very fine sand were likewise combined and listed as fine sand. The silt or particles having a diameter between 0.005 to

0.05 mm. were separated from the clay particles in the usual manner and dried at room temperature. The clay or particles less than 0.005 mm. in diameter were obtained by transferring the soil solutions containing the clay in suspension to large shallow dishes and evaporating to dryness in the sunlight. Soil 11076 contained a very small amount of coarse sand which was added to the fine sand separate. The percentages obtained by this classification are given in table 2.

TABLE 1
Lime requirements of soils by two methods
Pounds of CaCO_3 per 2,000,000 pounds of soil

NUMBER	SOIL TYPE	VEITCH METHOD	JONES METHOD
		pounds	pounds
11076	Willamette silt loam.....	3,200	2,620
11077	Salem gravelly loam.....	1,500	2,160
11079	Clay loam.....	10,000	6,200
11080	Medium sandy loam.....	20,800	12,600

TABLE 2
The percentages of different separates in soils

NUMBER	COARSE SAND	FINE SAND	SILT	CLAY
	per cent	per cent	per cent	per cent
11076		28.0	52.1	19.9
11077	25.6	42.0	22.5	9.9
11079	28.5	26.5	29.2	15.8
11080	38.3	29.4	23.7	8.6

Five different salts, namely potassium nitrate, potassium chloride, potassium acetate, calcium acetate and sodium chloride, were used in the study. Approximately 0.1 *N* solutions of each of these salts were prepared. The exact amount of salt in 50 cc. of the 0.1 *N* solutions was determined by evaporating to dryness on the steam bath and finally to constant weight in the electric oven at 105°C. The amounts found were as follows:

KNO_3	0.4854
KCl	0.3745
$\text{K}(\text{C}_2\text{H}_3\text{O}_2)$	0.5024
$\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$	0.3900
NaCl	0.2900

Of the salt solutions prepared above 150-cc. portions were transferred to 250-cc. bottles and 15 gm. of the various soil separates were added to each respectively. The bottles were then agitated in a mechanical soil shaker for 3 hours and centrifuged at a high speed until all soil particles had settled and the supernatant liquid was clear. Aliquots of 50 cc. were then taken for

titration and for the determination of the salt content. The acidity liberated by the various salt solutions was determined by titration with 0.04 *N* sodium hydroxide with phenolphthalein indicator. Table 3 gives the number of cubic centimeters of 0.04 *N* sodium hydroxide that was required to neutralize the acidity of the soil separates liberated by the different salt solutions.

The 50-cc. portions taken for the determination of the salt content were evaporated to dryness in a flat-bottom dish and dried to constant weight in

TABLE 3

The amount of NaOH required to neutralize the acidity in 50 cc., employing the 0.1 N salt solution designated

SOIL NUMBER	SEPARATE	K ($C_2H_3O_2$)	KCl	KNO ₃	Ca($C_2H_3O_2$) ₂	NaCl
		cc.	cc.	cc.	cc.	cc.
11076	Sand	2.50	0.03	0.03	3.00	0.03
11076	Silt	2.50	0.03	0.03	2.70	0.03
11076	Clay	4.50	0.20	0.24	5.30	0.10
11077	Coarse sand	2.00	0.06	0.06	2.44	0.03
11077	Fine sand	1.30	0.03	0.03	1.80	0.03
11077	Silt	2.44	0.14	0.06	3.10	0.03
11077	Clay	1.82	0.40	0.40	2.30	0.20
11079	Coarse sand	4.80	0.20	0.22	5.00	0.11
11079	Fine sand	4.50	0.20	0.20	4.60	0.11
11079	Silt	5.50	0.22	0.22	5.60	0.12
11079	Clay	5.20	0.38	0.40	5.00	0.22
11080	Coarse sand	11.42	1.80	1.40	12.00	0.42
11080	Fine sand	11.30	1.42	1.82	11.40	0.40
11080	Silt	9.80	1.36	1.50	9.40	0.42
11080	Clay	10.40	1.10	1.00	10.60	0.68

the electric oven at 105°C. Table 4 reports the actual amount of salt contained in 50 cc. of the salt solution used, the amount found after contact with the soil separates and the differences, that is, whether there was a greater or less amount of salt after contact with the soil separates than before. The plus (+) sign indicates that the quantity of salt in 50 cc. after contact with the separates was greater by the amount designated, than the amount contained in 50 cc. of the prepared solution; the minus (−) sign indicates the opposite to the plus sign, that is, a decrease in the amount found after contact with the soil particles.

TABLE 4

The amount of salts found in 50 cc. of solution before and after contact with soil separates

SOIL NUM- BER	DESCRIPTION	POTASSIUM CHLORIDE			SODIUM CHLORIDE					
		Amount used	Amount found	Difference	Amount used	Amount found	Difference			
		gm.	gm.	gm.	gm.	gm.	gm.			
11076	Sand	0.3745	0.4170	0.0425+	0.2933	0.3456	0.0523+			
11076	Silt	0.3745	0.4118	0.0373+	0.2933	0.3320	0.0387+			
11076	Clay	0.3745	0.4160	0.0415+	0.2933	0.3344	0.0411+			
11077	Coarse sand	0.3745	0.4070	0.0325+	0.2933	0.3176	0.0243+			
11077	Fine sand	0.3745	0.4036	0.0291+	0.2933	0.2972	0.0039+			
11077	Silt	0.3745	0.3794	0.0049+	0.2933	0.2990	0.0057+			
11077	Clay	0.3745	0.3988	0.0243+	0.2933	0.3300	0.0367+			
11079	Coarse sand	0.3745	0.3868	0.0123+	0.2933	0.3398	0.0435+			
11079	Fine sand	0.3745	0.3984	0.0239+	0.2933	0.3200	0.0267+			
11079	Silt	0.3745	0.3998	0.0253+	0.2933	0.3250	0.0317+			
11079	Clay	0.3745	0.4140	0.0395+	0.2933	0.3310	0.0377+			
11080	Coarse sand	0.3745	0.3942	0.0207+	0.2933	0.3256	0.0323+			
11080	Fine sand	0.3745	0.3878	0.0133+	0.2933	0.3134	0.0201+			
11080	Silt	0.3745	0.4000	0.0245+	0.2933	0.3184	0.0251+			
11080	Clay	0.3745	0.4270	0.0475+	0.2933	0.3482	0.0549+			
		POTASSIUM ACETATE			CALCIUM ACETATE			POTASSIUM NITRATE		
		Amount used	Amount found	Difference	Amount used	Amount found	Difference	Amount used	Amount found	Difference
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
11076	Sand	0.4854	0.5066	0.0112+	0.3900	0.3926	0.0026+	0.5024	0.5100	0.0076+
11076	Silt	0.4854	0.4824	0.0016-	0.3900	0.3960	0.0060+	0.5024	0.5240	0.0216+
11076	Clay	0.4854	0.4806	0.0048-	0.3900	0.3832	0.0068-	0.5024	0.5150	0.0126+
11077	Coarse sand	0.4854	0.5046	0.0192+	0.3900	0.3814	0.0086-	0.5024	0.5160	0.0136+
11077	Fine sand	0.4854	0.5038	0.0184+	0.3900	0.3866	0.0034-	0.5024	0.5146	0.0122+
11077	Silt	0.4854	0.4828	0.0026-	0.3900	0.3856	0.0044-	0.5024	0.5040	0.0016+
11077	Clay	0.4854	0.4830	0.0024-	0.3900	0.3878	0.0022-	0.5024	0.5232	0.0232+
11079	Coarse sand	0.4854	0.4678	0.0176-	0.3900	0.3774	0.0126-	0.5024	0.5110	0.0086+
11079	Fine sand	0.4854	0.4814	0.0040-	0.3900	0.3802	0.0098-	0.5024	0.5116	0.0092+
11079	Silt	0.4854	0.4772	0.0082-	0.3900	0.3788	0.0112-	0.5024	0.5160	0.0136+
11079	Clay	0.4854	0.5106	0.0252+	0.3900	0.3836	0.0064-	0.5024	0.5230	0.0206+
11080	Coarse sand	0.4854	0.4646	0.0208-	0.3900	0.3628	0.0272-	0.5024	0.5038	0.0014+
11080	Fine sand	0.4854	0.4652	0.0202-	0.3900	0.3592	0.0318-	0.5024	0.4932	0.0092-
11080	Silt	0.4854	0.4762	0.0092-	0.3900	0.3636	0.0364-	0.5024	0.4944	0.0080-
11080	Clay	0.4854	0.4756	0.0098-	0.3900	0.3891	0.0009-	0.5024	0.5264	0.0240+

DISCUSSION

According to the results given in table 3, the acidity of the various separates of a certain soil was slightly different but the different salt solutions showed a wide range in the amount of acidity liberated. The greatest amount of acidity was set free by the salts of an organic acid, namely, potassium acetate and calcium acetate, while the salts of the inorganic acids, potassium nitrate, potassium chloride, and sodium chloride, gave much lower results. Similar observations have been made by other workers when salt solutions and the soil as a whole were used. It is interesting to note that the smaller particles or the clay separates which may be assumed to contain most of the soil colloids and organic matter, did not show a much higher acidity and in several cases less acidity than the coarse sand separates.

In table 4 we observe that the various salt solutions have a widely different effect on the soil separates as judged by the amount of the salt in solution after contact with the soil fractions. Here again we see a distinct difference between the action of salts of the organic and inorganic acids. Calcium-acetate and potassium-acetate solutions contained in almost every case a lower salt content after contact than before contact with the soil separates. The salts of the mineral acids, on the other hand, gave an increased amount after treatment with the soil particles. This was contrary to the results reported by Parker (3) in his work on selective adsorption by soils where he found a loss in salt content after contact with the soil. Furthermore, sodium chloride, although liberating the smallest amount of acidity, gave the highest salt content in solution after contact with the soil particles. In order to ascertain if possible the reason for the variation in the acidity of the soil separates, the causes for the difference in salt content of a solution after contact with the soil separates, and the manner in which the salts of organic and inorganic acids react, the experiment was repeated, and close observation of any significant reaction noted. Besides using 0.1 *N* salt solutions, normal solutions of the salts also were prepared and used in a similar manner to the weaker solutions. The salt content of the normal solutions was not determined after contact with the soil particles, since it was thought that the solvent effects and reaction of the stronger solutions on the soil particles would be so great that no reliable inferences could be drawn therefrom. Table 5 gives the number of cubic centimeters of 0.04 *N* NaOH required to neutralize the acidity liberated by normal salt solutions.

The presence of aluminum in a salt extract of an acid soil has long been known and reported by many workers. Likewise, during the titration of the potassium-chloride and potassium-nitrate solutions with sodium hydroxide after contact with the soil particles, it was observed that an appreciable amount of aluminum hydroxide and in most instances small amounts of iron hydroxide were precipitated out. The amounts varied somewhat for the separates of the different soils and may be summed up as follows: no. 11076

gave no visible amount of iron hydroxide; no. 11077, on the other hand, showed larger amounts of iron than aluminum, the quantity of the former varying somewhat in the different separates; the precipitate from no. 11079 and 11080 consisted chiefly of aluminum hydroxide with varying amounts of iron hydroxide in the separates. These observations were substantiated by the qualitative colorimetric method recently suggested by Comber (1) in which the characteristic red color of ferric thiocyanate was developed by treating the soil separates with alcoholic potassium-thiocyanate solution, the potassium of the potassium thiocyanate, displacing the iron which subsequently formed the color reaction. Furthermore, potassium-nitrate and chloride solutions after contact with soil 11080 showed only a slight acidity

TABLE 5

The amount of NaOH required to neutralize the acidity in 50 cc., employing the 1.0 N salt designated

SOIL NUMBER	SEPARATE	K(C ₂ H ₃ O ₂)	KCl	KNO ₃	Ca(C ₂ H ₃ O ₂) ₂
		cc.	cc.	cc.	cc.
11076	Sand	4.40	0.20		5.60
11076	Silt	4.00	0.10		5.00
11076	Clay	10.70	0.16		5.24
11077	Coarse sand	4.30	0.03		7.40
11077	Fine sand	2.40	0.03		4.30
11077	Silt	5.10	0.03		8.00
11079	Coarse sand	11.20	0.90	0.70	10.26
11079	Fine sand	9.90	0.90	0.70	10.40
11079	Silt	12.86	0.90	0.75	13.16
11079	Clay	12.30	0.94	0.72	
11080	Coarse sand	20.80	5.20	4.82	18.50
11080	Fine sand	19.40	4.12	4.80	19.10
11080	Silt	24.50	5.00	4.86	16 10

to methyl orange indicator, while after contact with the other soils the salt solutions were apparently neutral. Since salt solutions of aluminum chloride and aluminum nitrate are neutral to methyl orange and acid to phenolphthalein and appreciable amounts are present in the salt solution after contact with the separates, it is evident that the acidity liberated by salts of inorganic acids of the soil under observation is due to basic exchange in which the stronger basic elements, potassium and sodium, displace iron and aluminum and the latter pass into solution as nitrate or chloride depending upon the salt used for extraction, and may subsequently be titrated with sodium hydroxide, with phenolphthalein as an indicator. This conclusion was further substantiated by means of Truog's (6) zinc-sulfide method for acidity. It was observed in the Truog test for acidity in which calcium chloride and zinc sulfide is used and the degree of acidity present estimated by the intensity

of coloration of lead acetate paper, that the salts aluminum nitrate, aluminum chloride and ferric chloride gave similar indications of acidity depending upon the amount of salt used. Consequently, comparisons were made between the intensity of color produced by an amount of aluminum nitrate, approximately equal to the quantity of aluminum and iron hydroxide liberated from soil 11079 by a normal solution of potassium nitrate, and the intensity of color produced by the acidity of the soil as a whole. The intensity of the color produced by the aluminum nitrate and by the soil was approximately the same.

The chemical composition of the soil separates and the distribution of organic matter and soil colloids in the different separates may be factors that would influence the results reported in the tables above and thus account for any variation that appears abnormal. In a consideration of these points it may be assumed that most of the soil colloids are present in the finest particles or clay separates. The chemical composition of soil separates prepared

TABLE 6
The average amount of several constituents in soil separates

CONSTITUENT	SAND	SILT	CLAY
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SiO ₂	88.50	83.05	45.52
Fe ₂ O ₃	1.66	1.96	8.73
Al ₂ O ₃	5.48	8.44	22.57
CaO.....	0.59	0.48	0.64

TABLE 7
Percentage of organic matter in the separates of a soil

SOIL NUMBER	COARSE SAND	FINE SAND	SILT	CLAY
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
11079	4.60	3.67	5.95	6.06

from ten different samples has been investigated by Steinkoenig (5). The average amounts of silicon oxide, iron oxide, aluminum oxide and calcium oxide found in the various soil separates are given in table 6.

The results indicate that the large particles or sand separates contain the highest amount of silicon oxide while the finer or silt separates contain higher amounts of calcium, iron and aluminum oxides. Failyer *et al* (2) report greater amounts of lime, magnesia, potash and phosphoric acid in the finer particles or clay separates, which was confirmed also by Steinkoenig (5). In regard to the distribution of organic matter it is probable that larger amounts will be found in the finer particles or clay separate. In order to confirm this assumption a determination of organic matter in the separates of soil 11079 was made. The Rather (4) hydrogen fluoride method was followed. The results are given in table 7.

The very fine particles or the clay and silt separates contain approximately the same amount of organic matter. The coarse sand, apparently, contains a higher amount of organic matter than the fine sand but this is probably due to the fact that small roots and other debris that were too large to be classified with the very fine sand were present in visible quantities in the coarse sand. When we consider, therefore, the differences in the composition of the various soil separates, the unequal distribution of colloids and organic matter, the molecular weight of the elements that take part in the basic exchange, and the solvent effect of the salt solutions, it is obvious that the slight variation in the acidity of the soil separates and the variation in the amount of salt found after contact with the separates, may be explained.

From the results obtained with the salts of the organic acid, namely, calcium acetate and potassium acetate, it is apparent that a different reaction has taken place from that indicated by the salts of mineral acids. A far greater amount of acidity was liberated while there was a decrease in the salt content of 50 cc. after contact with the soil separates. If the reaction had been similar, but more intense as indicated by higher acidity, to the reaction of the salts of mineral acids, a large increase in the salt content of the 50 cc. would be expected after contact with the soil separates. On account of the dissimilarity of the reactions another explanation was sought.

Examinations of the organic salt solutions after contact with the soil separates did not show the presence of iron and aluminum hydroxide when neutralized with sodium hydroxide, as was evident with the salts of inorganic acids. A determination of the calcium content of the calcium-acetate solution before and after contact showed that appreciable quantities of calcium had been taken up by the soil but no metallic elements had been displaced in the reactions. Furthermore, since there was a decrease in the salt content after reaction with the soil, it was thought that the calcium may have been selectively adsorbed or by basic exchange had replaced the hydrogen of hydrous silicates. In either case free acetic acid would be formed and by distillation could be quantitatively determined. Accordingly, 20 gm. of soil 11079 was introduced into a bottle containing 200 cc. of calcium-acetate solution, the calcium content of which had been accurately determined. The bottle was then shaken in a mechanical shaker for 3 hours, centrifuged to clarify the solution and filtered. Fifty-cubic-centimeter portions of the clear solution were then taken for the determination of the acidity liberated as indicated by titration immediately with sodium hydroxide, with phenolphthalein as an indicator, and 50 cc. for distillation which would give the acetic acid present. The distillation was allowed to proceed nearly to dryness and the distillate titrated with sodium hydroxide, with phenolphthalein as an indicator. Also 25-cc. aliquots were taken and the calcium content determined and calculated to calcium acetate. The results are given in table 8.

The results show that the calcium taken up by the soil from the calcium-acetate solution was equivalent approximately to the acetic acid liberated.

Furthermore, we see that the acidity of the distillate was the same as the acidity of the salt solution that had not been distilled. This indicates that all of the acidity liberated in the reaction was acetic acid. Considered from this standpoint, the reason for an actual decrease in the salt content of the organic salt solutions after reaction with the soil particles, can be understood. The calcium was removed from the salt solution and was replaced by hydro-

TABLE 8

Amount of calcium acetate in solution before and after contact with soil and the calcium acetate equivalent of the acetic acid liberated

SOIL NUMBER	BEFORE CONTACT	AFTER CONTACT	DIFFERENCE	ACID LIBERATED	ACIDITY OF DISTILLATE
	gm.	gm.	gm.	gm.	gm.
11079	0.3520	0.2982	0.0538	0.0484 (12.27 cc. 0.05 N NaOH)	0.0479 (12.12 cc. 0.05 N NaOH)

TABLE 9

H⁺ ion concentration of soil separates

SOIL NUMBER	SEPARATE	E $\frac{N}{10}$	pH
11076	Sand	0.650	5.31
11076	Silt	0.650	5.31
11076	Clay	0.654	5.38
11077	Coarse sand	0.696	6.09
11077	Fine sand	0.686	5.92
11077	Silt	0.683	5.87
11077	Clay	0.683	5.87
11079	Coarse sand	0.658	5.44
11079	Fine sand	0.640	5.14
11079	Silt	0.668	5.61
11079	Clay	0.666	5.58
11080	Coarse sand	0.642	5.17
11080	Fine sand	0.644	5.21
11080	Silt	0.642	5.17
11080	Clay	0.640	5.14

gen. The difference in the atomic weight of calcium and hydrogen would necessarily result in a smaller salt content even though the acetic acid were not removed during the evaporation process.

The actual acidity of the soil separates as indicated by the hydrogen-ion concentration was determined by the gas-chain method. Three-gram portions of the different soil separates were transferred to small bottles that served as the electrode vessel. Two cubic centimeters of conductivity water was added to each separate and allowed to stand 24 hours. Immediately

before the determination was made, more water was added to obtain 3 gm. of soil to 30 cc. of water. The increase in potential due to the H^+ ion, was obtained by taking readings directly from a millivoltmeter. The voltage at 25° , $E \frac{N_i}{10}$, and the Sorensen pH values are given in table 9.

The results indicate that the hydrogen-ion concentration of different separates of the soils under observation is approximately the same. Apparently, therefore, the size of the soil particles, the presence of colloids or organic matter in the clay separates or the composition of the different separates does not have a great influence on the hydrogen-ion concentration.

SUMMARY

A study of the action of several salt solutions on the soil separates has been made.

The acidity of the different soil separates liberated by action of a certain salt solution is approximately the same.

The manner in which the salts of mineral acids, KNO_3 , KCl and $NaCl$ react with the soils studied, is apparently different from the salts of an organic acid, $K(C_2H_3O_2)$ and $Ca(C_2H_3O_2)_2$.

The so-called acidity liberated by potassium nitrate, potassium chloride, and sodium chloride was due mainly to aluminum and iron rendered soluble by basic exchange.

The acidity produced by calcium acetate and potassium acetate was due to acetic acid liberated either by replacement of the hydrogen of hydrous silicates or by selective adsorption of the basic element in the salt solution.

The hydrogen-ion concentration of different separates of the soil was constant.

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ACID SOIL STUDIES: II. CHANGES IN CALCIUM COMPOUNDS ADDED TO ACID SOILS

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This paper deals with another phase of the problem reported in the foregoing article (2) in which studies were made to ascertain, if possible, the reasons why some acid soils of Oregon do not respond to lime treatment. The question now arises regarding the changes that calcium carbonate undergoes, when applied to an acid soil, if it does not function in correcting the acid condition; or if it does neutralize the acidity, in what manner is the reaction or chemical combination different from that exhibited by calcium carbonate applied to an acid soil that does respond to lime treatment? In an effort to answer this question a study was made of the changes that occurred when certain calcium compounds were added to the two types of acid soils.

The same so-called acid soils were used in this work as in the study reported in the foregoing paper (2). The same laboratory numbers, namely 11076, 11077, 11079 and 11080 were maintained. Further information regarding classification, lime requirement, or other points in question may be ascertained by reference to paper I (2).

A sufficient amount of each of the different soils was prepared to fill pots having a capacity of approximately 3 gallons. Chemically pure calcium carbonate and calcium oxide, prepared by igniting pure calcium carbonate, were used in amounts equivalent to the lime requirement of the different soils. Soil 11077 which, according to field observations, did not respond to lime treatment, received additional treatments in which calcium sulfate was used in amounts equivalent to the lime requirement and calcium carbonate was used in an amount that was double the lime requirement. After thoroughly incorporating the calcium compounds with the dry soil it was transferred to the pots. Control pots containing each of the different soils untreated also were prepared. Table 1 gives the amounts of different soils used and the treatments.

The pots containing the soil treated in the manner described above were then sunk into the ground level with the top of the pots and exposed to the weather. A crop of spring barley was grown in all pots to aid natural functioning and changes that the calcium compounds might undergo.

After exposure to the weather for one year, representative samples, taken to the depth of 6 inches, were removed from each pot. The forms into which

the calcium compounds had changed were then determined by the methods suggested by Shorey, Fry and Hazen (3). The methods, changed to suit the work at hand, are as follows:

The total calcium was determined by decomposing 5 gm. of soil with 10 gm. of sodium peroxide at low heat. The mass was then taken up with water, acidified, and the calcium precipitated as oxalate in an aliquot after the removal of iron and aluminum.

The carbon dioxide was liberated and absorbed in barium hydroxide according to the method outlined by Truog (4).

Water-soluble calcium oxide and sulfur trioxide were determined in the water extract obtained by shaking 40 gm. of soil with 200 cc. of water and centrifuging at a high speed until a clear solution was obtained.

TABLE 1
Treatment of soils

SOIL NUMBER	WEIGHT OF SOIL USED	COMPOUND ADDED	AMOUNT ADDED
	gm.		gm.
11076	12000	Control	
11076	13000	CaCO ₃	21.8
11076	12800	CaO	12.9
11077	12500	Control	
11077	12500	CaCO ₃	11.4
11077	12000	CaCO ₃	22.3
11077	12000	CaO	6.2
11077	12000	CaSO ₄ ·2H ₂ O	19.1
11079	12700	Control	
11079	12700	CaCO ₃	65.3
11079	12700	CaO	37.2
11080	11000	Control	
11080	11000	CaCO ₃	59.6
11080	11000	CaO	33.5

The acid-soluble calcium was determined by two methods designated "A" and "B," respectively, by Shorey, Fry, and Hazen (3). In method "A" the soil was digested with 4 per cent hydrochloric acid; in method "B" it was leached with 2 per cent hydrochloric acid. After washing the soil free of acid the calcium was determined in the respective filtrates.

The data obtained by these methods are given in table 2.

The interpretation of the analytical data reported above in terms of calcium compounds formed assume certain combinations. The schematic presentation and system of calculation as employed by Shorey, Fry and Hazen (3) was followed. Table 3 gives the percentages of various hypothetical calcium compounds that were present in the treated and in the control soils.

TABLE 2

The percentages of constituents obtained by the method employed

SOIL NUMBER	TREATMENT	TOTAL CaO	ACID-SOLUBLE CaO		WATER-SOLUBLE CaO	WATER-SOLUBLE SO ₂	CARBON DIOXIDE
			4 per cent HCl	2 per cent HCl			
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
11076	Control	1.05	0.37	0.38	0.012	0.008	Negative
11076	CaCO ₃	1.12	0.51	0.47	0.013	0.006	Negative
11076	CaO	1.12	0.45	0.45	0.020	0.009	Negative
11077	Control	2.60	0.20	0.10	0.015	0.004	Negative
11077	CaCO ₃ (11.4 gm.)	2.68	0.46	0.13	0.018	0.005	Negative
11077	CaCO ₃ (22.3 gm.)	2.75	0.69	0.16	0.020	0.004	Negative
11077	CaO	2.76	0.34	0.10	0.020	0.005	Negative
11077	CaSO ₄	2.75	0.49	0.10	0.017	0.005	Negative
11079	Control	0.27	0.18	0.17	0.018	0.009	Negative
11079	CaCO ₃	0.60	0.45	0.45	0.018	0.007	Negative
11079	CaO	0.56	0.34	0.33	0.013	0.008	Negative
11080	Control	2.06	0.45	0.43	0.022	0.014	Negative
11080	CaCO ₃	2.45	0.89	0.60	0.030	0.012	Negative
11080	CaO	2.45	0.79	0.59	0.039	0.007	Negative

TABLE 3

Hypothetical calcium combinations in treated and untreated soils

SOIL NUMBER	TREATMENT	TOTAL CaO	CaO AS CaCO ₃	CaO AS EASILY DECOMPOSABLE SILICATE	CaO AS DIFFICULTLY DECOMPOSABLE SILICATE	CaO WITH HUMUS	CaO AS CaSO ₄
		<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
11076	Control	1.05	None	0.00	0.68	0.370	0.010
11076	CaCO ₃	1.12	None	0.04	0.62	0.460	0.010
11076	CaO	1.12	None	0.00	0.67	0.440	0.010
11077	Control	2.60	None	0.11	2.40	0.084	0.006
11077	CaCO ₃ (11.4 gm.)	2.68	None	0.33	2.22	0.123	0.007
11077	CaCO ₃ (22.3 gm.)	2.75	None	0.53	2.06	0.154	0.004
11077	CaO	2.76	None	0.24	2.42	0.095	0.007
11077	CaSO ₄	2.75	None	0.39	2.26	0.095	0.007
11079	Control	0.27	None	0.01	0.09	0.160	0.010
11079	CaCO ₃	0.60	None	0.00	0.15	0.440	0.010
11079	CaO	0.56	None	0.01	0.22	0.320	0.010
11080	Control	2.06	None	0.02	1.61	0.410	0.020
11080	CaCO ₃	2.45	None	0.29	1.56	0.580	0.020
11080	CaO	2.45	None	0.20	1.46	0.580	0.010

A comparison of the total calcium-oxide content of the treated soils with the total calcium oxide of the controls shows that most of the calcium had been retained, although no doubt part had been lost by leaching since about 40 inches of rainfall had occurred during the year. Furthermore, there was a complete change to other combinations from the form in which the calcium had been added. In no case was any calcium carbonate present as indicated by the negative result for carbon dioxide. In the soil that had been treated with calcium sulfate no increase in water-soluble sulfur trioxide was obtained, which indicates that all sulfates had been lost by leaching. Since, however, some of the calcium had been retained by the soil, it is probable that as the calcium sulfate dissolved, the calcium that was retained was taken up by the soil by basic exchange, while the sulfate was leached out in the substituted form.

TABLE 4

Acidity, by the Jones method, of treated and untreated soils after exposure to weather for one year

SOIL NUMBER	TREATMENT	0.04 N NaOH
		cc.
11076	Control	3.20
11076	CaCO ₃	1.70
11076	CaO	2.10
11077	Control	3.00
11077	CaCO ₃ (11.4 gm.)	2.40
11077	CaCO ₃ (22.3 gm.)	1.53
11077	CaO	2.45
11077	CaSO ₄	3.00
11079	Control	6.80
11079	CaCO ₃	2.70
11079	CaO	3.60
11080	Control	12.90
11080	CaCO ₃	4.10
11080	CaO	4.30

With the exception of soil 11077, most of the calcium added to the different soils was combined with humus. Since in most cases there was a higher water-soluble calcium content in the treated soils than in the controls, it is apparent that the calcium with humus would probably be more easily available and would provide an optimum medium for the development of favorable soil organisms. On the other hand soil 11077 which does not respond to lime treatment, showed that very little of the added calcium combined with humus, but was used to form easily decomposable silicates that are soluble in 4 per cent hydrochloric acid but not in 2 per cent hydrochloric acid. Where calcium was added to this soil in an amount that was double the lime requirement, more of the calcium combined with humus.

There was no marked difference between the reactions of calcium carbonate and calcium oxide or in the compounds formed.

Observations were made on the change in the lime requirement as indicated by the Jones (1) method for soil acidity. Table 4 reports the acidity liberated, in terms of cubic centimeters of 0.04 *N* sodium hydroxide.

The results indicate a reaction in which acid is liberated, although an excess of calcium carbonate and calcium oxide had been added to the treated soils. It is noticeable in soil 11077 where 22.3 gm of calcium carbonate, or double the lime requirement, had been added that the acid liberated was lower than where 11.4 gm had been added.

Since the Veitch (5) method was employed as a criterion of the lime requirement, tests were made to ascertain whether the soils after treatment and exposure to the weather for one year would react acid, as indicated by the Jones method. It was found that for every soil treated with calcium carbonate or calcium oxide an alkaline reaction was obtained. It is evident, therefore, that since water extracts of the treated soils were alkaline as determined by the Veitch test, the so-called acidity of these soils may be considered neutralized after exposure to the weather for one year.

CONCLUSIONS

When calcium carbonate or calcium oxide was added to several acid soils the calcium retained after exposure to the weather for one year was combined chiefly with humus (organic matter) and easily decomposable silicate.

Most of the calcium present in the acid soil that does not respond to lime treatment was found combined as difficultly decomposable silicate. The calcium added was combined chiefly as easily decomposable silicate. This, however, does not explain the reason why the soil does not respond to lime treatment.

After exposure to the weather for one year all of the soils treated with either calcium carbonate or calcium oxide were alkaline according to the Veitch test.

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THE INFLUENCE OF FERTILIZERS CONTAINING BORAX ON THE YIELD OF POTATOES AND CORN—SEASON 1920¹

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Several reports (3, 5)² have appeared concerning injury to the potato crop, during the season of 1919, by borax which occurred as an impurity in certain commercial potash salts obtained chiefly from Searles Lake, Cal. Reports (1, 2, 4) also have been issued concerning injury to other crops the same season, along with reports of investigational studies on the subject.

As the result of certain control measures, as well as careful supervision on the part of the companies producing the potash salts, no authentic case, so far as known, of injury to crops by borax was reported in 1920.

Certain points having arisen, however, it seemed desirable to conduct field investigations in order to ascertain: (a) the effects of different concentrations of borax upon the growth and yield of a number of crop-plants; (b) the influence of rainfall and soil type upon the effect of borax; and (c) the influence of time and method of application of a fertilizer mixture containing added borax in varying concentrations, upon the growth and yield of the selected crop-plants.

Following out this idea the Bureau of Plant Industry of the U. S. Department of Agriculture, through Dr. Oswald Schreiner, in charge of Soil Fertility Investigations,³ asked the New Jersey Agricultural Experiment Station to coöperate in some experiments in which varying amounts of borax should be introduced in a control fertilizer, to be used on potatoes and corn.

The control fertilizer was a mixture of cottonseed meal, acid phosphate and muriate of potash analyzing 4 per cent ammonia, 8 per cent phosphoric acid and 4 per cent potash. The borax was mixed with this fertilizer in such proportions as to make the anhydrous borax application, in pounds per acre, as

¹ Paper No. 20 of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology, cooperating with the United States Department of Agriculture.

² Also articles appearing in the agricultural press.

³ Other cooperative stations were established in Maine, Virginia, and Alabama under the direction of the Office of Soil Fertility Investigations.

follows: 1, 2, 3, 4, 5, 10, 20, 50, 100, 200 and 400. To simplify the work, the plot number was made to correspond with the number of pounds of borax per acre; thus plot 1 corresponds to 1 pound of borax with 1500 pounds of the standard fertilizer per acre; plot 2 to 2 pounds of borax and 1500 pounds of fertilizer per acre, etc. Each plot consisted of two rows of potatoes (one row of corn) the full length of the strip.

Check rows, that is, those which receive fertilizer without borax, were introduced as follows: check 1 outside of plot 1; check 2, between plots 3 and 4; check 3, between plots 10 and 20, and check 4 between plots 50 and 100. This arrangement provided a fair distribution of checks over the area and thus made it possible to interpret results even though there is some lack of uniformity in the soil.

The soil type on which this experiment for both corn and potatoes was conducted is a Sassafras loam of good quality.

In order that a test might be made of the influence of time and method of applying the fertilizers, the plot of ground was divided crosswise into three equal sections with the provision that the fertilizer should be drilled in the furrow on section 1 some two or three weeks before planting the crop; on section 2 it was to be drilled in the furrow at the time of planting, and on section 3 spread broadcast over the furrow at the time of planting.

THE POTATO CROP

For the potatoes the fertilizer was applied in the furrow by hand to section 1 on April 16; it was slightly mixed with the soil by passing a hoe along the bottom of the furrow. It was left in this condition until April 27 when the applications were made to sections 2 and 3 as above noted and potatoes planted on all sections. On sections 2 and 3 the fertilizer was slightly mixed with the soil, as in the case of section 1, before the potatoes were dropped. Covering was done by means of a horse cultivator.

This method of applying fertilizer and planting, it was thought, would clear up the question as to whether early application of the fertilizer might partially or wholly overcome the injurious action of the borax, which had been noted when fertilizer was applied at the time of planting the potatoes.

With the exceptions noted, that is, the time and method of applying the fertilizers, the three sections were treated exactly alike. The potatoes received the usual attention in the way of cultivation, hoeing and spraying. They were kept free from grass and weeds until toward the end of the season when, on account of much rain, grass made considerable headway on all plots except those which received the heavy applications of borax.

The weights of the potatoes, by plots, for the three sections, are shown in table 1. The table is best studied section by section and since the "seconds" form a rather small percentage of the total weight, the conclusions will probably be the same whether one considers the weight of the "primes" or the total weights.

Section I

When the weights for the different plots are considered with reference to the check plot nearest any given plot, it would appear that there is little or no decrease in yield for this section with as much as 50 pounds of borax per acre. It may be pointed out that there is actually a decline in yield from check plot 3 on through the plots that received 20, 30 and 50 pounds of borax per acre, but check 4 which lies along side of the plot receiving 50 pounds of borax yielded a total of only 72.8 pounds of potatoes as against 71.5 pounds for the plot with 50 pounds of borax. From this, it would appear that the gradual decline in yield from check 3 to check 4 was due to some other factor

TABLE 1
Yield of potatoes in coöperative borax experiment

QUANTITY OF BORAX PER ACRE	SECTION 1			SECTION 2			SECTION 3		
	Primes	Seconds	Total	Primes	Seconds	Total	Primes	Seconds	Total
<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
0 (Check 1)	78.25	7.50	85.75	75.50	5.50	81.00	58.25	7.50	65.75
1	64.20	5.75	69.95	56.30	6.85	63.15	41.40	5.00	46.40
2	75.55	4.65	80.20	72.50	9.00	81.50	58.05	5.80	63.85
3	75.65	6.65	82.30	65.60	9.10	74.70	60.15	5.75	65.90
0 (Check 2)	79.75	7.70	87.45	62.95	10.35	73.30	65.70	4.45	70.15
4	88.35	6.40	94.75	69.55	7.60	77.15	66.35	4.95	71.30
5	85.20	6.00	91.20	77.15	4.65	80.80	62.20	8.00	70.20
10	88.60	5.90	94.50	64.55	6.45	71.00	60.80	5.10	65.90
0 (Check 3)	86.90	5.75	92.65	67.75	8.60	76.35	65.80	4.10	69.90
20	85.70	3.75	89.45	68.75	6.90	75.65	79.00	3.00	82.00
30	76.95	3.00	79.95	55.00	3.85	58.85	74.00	3.55	77.55
50	67.95	3.55	71.50	25.35	1.40	26.75	45.70	2.00	47.70
0 (Check 4)	65.15	7.65	72.80	60.35	6.00	66.35	66.85	6.55	73.40
100	43.75	3.00	46.75	2.00	1.00	3.00	1.25	0.50	1.75
200	22.50	1.25	23.75	None	0.125	0.125	None	None	None
400	4.00	0.50	4.50	None	None	None	None	None	None

than the borax. Indeed, if the yield of "primes" is considered rather than the total yield, it is found that the plot which received 50 pounds of borax yielded 2.8 pounds more than the adjoining check plot.

With an application of 100 pounds of borax per acre the yield is cut to approximately one-half the normal and with 200 pounds of borax to about one-quarter the normal. With the 400-pound application, the crop was practically a failure, being less than one-twentieth of the normal.

Section II

For this section there is no distinct depression in yield that can be attributed to the borax until the 30-pound application of borax is reached, the total yield with 20 pounds being within 0.7 pound of the yield on check plot 3

which adjoins this plot. The 30-pound application caused a drop in yield and the 50-pound quantity brought it down to about one-third of the normal. With the 100-pound application, the total yield of potatoes was only 3 pounds. The still heavier applications resulted in complete failure.

The low yields on plot 1 of this section must be attributed to some other cause than the borax.

Section III

The highest total yield for this section—82 pounds—was from the plot which received 20 pounds of borax and the next highest—77.5 pounds—from the plot which received 30 pounds of borax per acre. Check plot 4 which is separated from the plot receiving 30 pounds of borax by only one plot (2 rows) gave a total yield of 73.4 pounds. The 30-pound application of borax, therefore, cannot be said to have caused any depression in the yield. The 50-pound application brought the “primes” down to 45.7 pounds, the total yield for this plot being 47.7. The 100-pound application gave a total yield of less than 3 pounds and the 200 and 400-pound applications resulted in total failure.

The heavy applications of borax either prevented germination entirely or resulted in a long delay in germination. On the plots which received these heavy applications a few plants came through but were several weeks later than the plants on the plots not affected by the borax. These delayed plants grew slowly, were slender and lacked vigor, and were abnormal in color. In some respects the injury is similar to the injury caused by soils heavily charged with alkalis.

THE CORN CROP

The same general plan was followed for the corn as for the potatoes. However, each plot or treatment consisted of a single row instead of two rows, as in the case of the potatoes.

The fertilizer was used at the rate of 400 pounds per acre, but the amount of borax applied remained the same as for the potatoes.

The fertilizer for section 1 was drilled in the furrow by hand on April 30. On May 15 the fertilizer was applied to sections 2 and 3 in the same manner as in the case of the potatoes, and the corn was planted on the three sections. The rows were run 4.5 by 3.4 feet. For each section there were 14 hills to the row with five kernels to the hill.

In order that additional information might be secured with reference to the influence of the borax on the germination of the corn, three single kernels were dropped (at equal distances from one another) between the hills.

On June 3 a count was made of the stalks in the hill and also for the inter-hill planting. The results of this count are shown in table 2. From this table it will be noted that on sections 2 and 3 germination was somewhat depressed with as low as 5 and 10 pounds of borax per acre. On section 1 this depression is noted at 20 pounds per acre for the hill planting and at

30 pounds for the inter-hill planting. With 50 pounds of borax per acre on section 2 only 9 out of a possible 70 plants were found for the hill planting, and only one out of a possible 42 for the inter-hill planting.

The inter-hill plants were finally removed and the hills thinned to 3 plants (some hills had only 2 plants).

TABLE 2
Germination count June 3; hill and inter-hill planting, corn borax experiment

HILLS	HILL PLANTING			INTER-HILL PLANTING		
	Section 1	Section 2	Section 3	Section 1	Section 2	Section 3
0 (Check 1)	55	52	57	32	34	34
1	58	55	58	30	25	34
2	60	54	57	30	30	33
3	59	55	55	31	31	33
0 (Check 2)	56	54	54	26	31	27
4	50	45	54	27	27	26
5	44	42	49	28	18	31
10	52	36	42	28	16	24
0 (Check 3)	51	61	59	31	35	33
20	43	23	35	30	5	21
30	38	13	28	25	7	12
50	46	9	8	27	1	4
0 (Check 4)	52	53	52	27	28	28
100	32	2	7	18	1	4
200	8	0	0	6	0	0
400	0	0	0	4	0	0

TABLE 3
Air-dry weights of corn in borax experiment

PLOT	GRAIN			COBS			STALKS		
	Section 1	Section 2	Section 3	Section 1	Section 2	Section 3	Section 1	Section 2	Section 3
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Check 1	11.30	19.88	19.59	2.43	4.32	4.33	24.25	22.00	21.40
1	7.96	16.79	17.39	1.63	3.73	3.44	25.00	26.95	18.90
2	8.87	14.93	20.53	1.81	3.18	4.33	23.00	25.75	23.60
3	14.30	13.36	16.79	3.05	2.69	3.44	21.15	18.20	20.80
Check 2	13.47	13.00	13.91	2.81	2.35	2.82	19.30	18.55	20.20
4	9.82	12.00	11.97	2.07	2.50	2.36	14.60	17.50	17.05
5	8.79	8.60	9.97	2.00	1.65	2.04	14.20	12.55	13.80
10	10.85	9.47	11.73	2.30	1.97	2.61	14.85	15.90	16.70
Check 3	7.95	11.68	15.90	1.83	2.44	3.16	18.70	16.52	19.85
20	8.64	9.24	13.90	1.93	1.78	2.84	18.10	15.13	21.05
30	11.77	7.57	12.63	2.67	1.99	2.86	22.95	16.70	21.70
50	11.08	3.41	6.79	2.62	0.92	1.66	18.75	7.30	15.65
Check 4	10.55	19.12	20.90	2.23	4.27	4.90	22.50	24.35	31.50
100	6.44	0.62	2.82	1.76	0.19	0.86	20.90	1.70	9.40
200	1.23	0.00	0.00	0.32	0.00	0.00	2.60	0.00	0.00
400	0.40	0.00	0.00	0.15	0.00	0.00	2.70	0.00	0.00

The corn received the usual cultivation during the summer and was harvested on October 11. The stalks and ears for each plot in the three sections were weighed and the weights of the stalks at this time were taken as the final weights. The ears, however, were dried and the corn shelled so that the weights here reported are for the dry shelled corn. The weights of stalks and corn for the three sections are shown in table 3.

Section I

The weights for section 1 do not indicate any definite depression in yield up to and including 50 pounds of borax per acre, this plot having yielded about $\frac{1}{2}$ pound more than check plot 4 which adjoins it. With 100 pounds of borax and over the depression in yield is very pronounced. This is clearly indicated by figure 1, plate 4.

The low yields for the check plots of this section are apparently due to unfavorable soil conditions. The weights of the corn stalks for these check plots as compared with the corresponding weights for sections 2 and 3 would indicate that the stalk growth was about normal.

Section II

The yields on plots 1, 2, and 3 are less than the yield of check plot 1 but are more than the yield on check plot 2. It would, therefore, appear that the depression noted (as compared with check 1) is due to a soil condition rather than to the borax treatment.

Beginning with the 5-pound application there appears to be some depression in yield, though it may be pointed out that the yield was almost as much where 20 pounds of borax was used as where only 10 pounds was used. With 50 pounds of borax the yield was reduced to 3.41 pounds and with 100 pounds to 0.62 pounds, as compared with 19.2 pounds for the adjoining check. With the 200 and 400-pound applications the crop was a complete failure.

The appearance of section 2, including plots 20, 30, 50 and check 4 on July 6, is well illustrated by figure 2, plate 4. It will be noted that the few stalks which did survive, were gradually recovering from the set-back given by the borax.

Section III

For this section there is not positive indication of injury until the 4-pound application is reached and possibly not until the 5-pound application. Indeed what appears to be a depression with 5 pounds may be due to a soil condition since the yield with 10, 20 and 30 pounds is considerably higher than the yield with 5 pounds.

For the 50-pound application the yield was 6.79 pounds, whereas the yield on the adjoining check plot was 20.9 pounds. The 100-pound application gave a yield of 2.82 pounds. The larger applications resulted in complete failure.

RAINFALL RECORD

It is well known that the rainfall during any given season has an important bearing on crop yields, and that the effectiveness of fertilizer salts may vary widely depending upon whether there is much or little rain. The same would apply to other soluble salts as, for example, borax.

The rainfall at New Brunswick during the summer of 1920 was unusually heavy, as shown by the monthly record in comparison with the average for the same months during the last ten years. The monthly rainfall from April to September, inclusive, 1920, and the 10-year average—1910 to 1919—is shown in table 4.

In connection with the effect of the borax on the potatoes it may be pointed out that between April 16 and 27—the time that the fertilizer remained in the ground on section 1, preceding the planting of the potatoes—the rainfall was 2.01 inches. On the day following the planting of the potatoes there was a fall of 0.6 inch.

TABLE 4
Rainfall at New Brunswick, New Jersey

	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER
	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>	<i>inches</i>
1920	4.28	3.56	9.64	6.00	8.21	2.23
10 year average, 1910						
1919	3.66	3.85	3.52	4.67	5.07	2.95

It is not probable that a rainfall of 2 inches in 2 weeks, fairly distributed, would wash very much of the borax entirely out of reach of the potato tubers. It would, however, result in more or less diffusion of the borax through the soil, and this diffusion, together with a certain amount of colloid absorption, could no doubt, account for the smaller amount of injury on section 1, where the fertilizer was in the ground 2 weeks before the potatoes were planted, than on the other two sections.

Between April 30, when the fertilizer was applied to section 1 for corn, and May 15, when the corn was planted, there was a rainfall of 2.33 inches, fairly distributed over the period. As in the case of the potatoes this resulted in more or less diffusion of the borax through the soil with the result that germination was more nearly normal on this section than on sections 2 and 3, where the corn was planted at the time of applying the fertilizer.

Had the rainfall for the season been normal or below normal, instead of considerably above normal, it is possible that the 5, 10, 20, 30 and 50-pound applications of borax might have resulted in greater injury to both corn and potatoes.

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PLATE 1

FIG. 1. Comparing 5, 10 and 20 pounds of borax with check, 5

FIG. 2 Comparing 400, 200, and 100 pounds of borax on sections 1, 2 and 3



FIG. 1



FIG. 2

PLATE 2

FIG. 1. Comparing the yield of potatoes on check 3 with 30 pounds of borax on sections 1, 2 and 3.

FIG. 2. Comparing the yield of potatoes on check 4 with 50 pounds of borax on sections 1, 2 and 3.

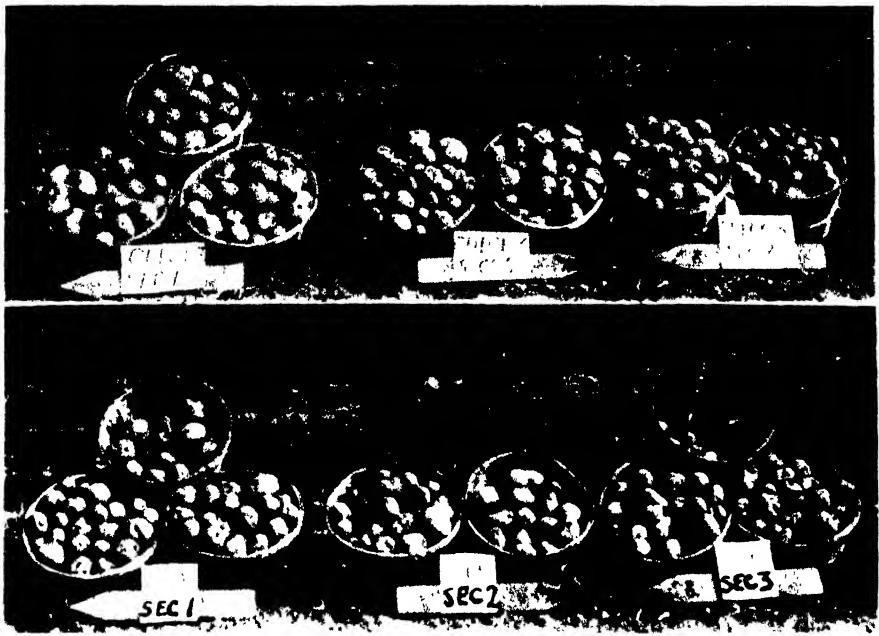


FIG. 1



FIG. 2

PLATE 3

FIG. 1. Comparing the yield of potatoes with 10 and 20 pounds of borax on sections 1, 2 and 3.

FIG. 2. Showing appearance of residual crop of rye where 100, 200 and 400 pounds of borax were used; no apparent injury to date.

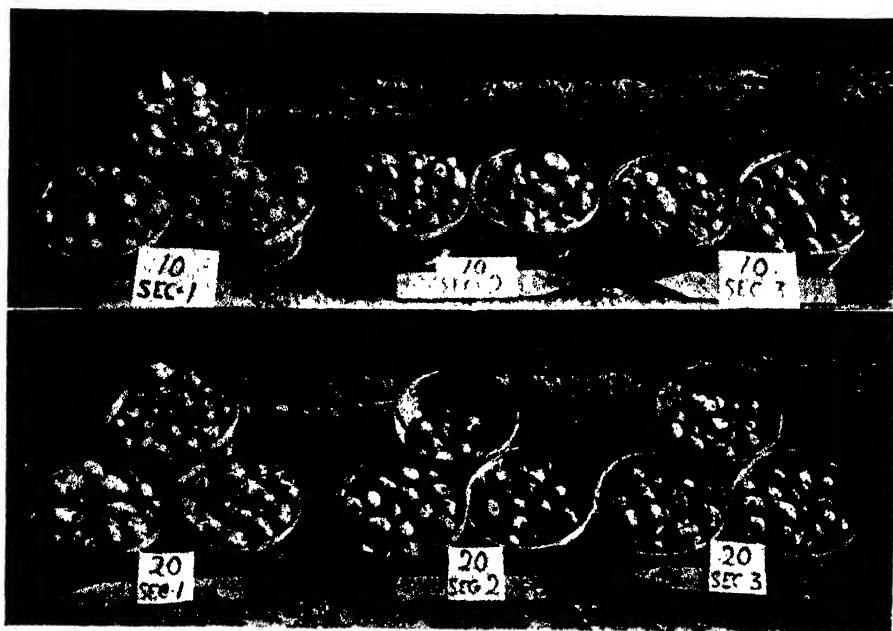


FIG. 1



FIG. 2

PLATE 4

FIG. 1. Showing corn on section 1 where 100, 200 and 400 pounds of borax were used, as compared with check 4.

FIG. 2. Showing corn on section 2, where 20, 30, and 50 pounds of borax were used, as compared with check 4.



FIG. 1



FIG. 2

SULFUR FOR NEUTRALIZING ALKALI SOIL¹

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Among numerous experiments with alkali soils made by the writer, some, in which sulfur was the active agent, seem of sufficient importance to be worthy of public notice.

About a year ago 1 per cent of sulfur was added to an alkali soil; to another portion of soil was added 1 per cent sulfur + 1 per cent alfalfa meal; to another portion 1 per cent sulfur + 1 per cent pulverized manure. With these was a jar of the soil without any treatment. Water was added to all four to bring the moisture content to 15 to 18 per cent. The jars of soil were covered loosely and kept in a storeroom for several months without attention. When examined, the three jars to which sulfur was added were found to be quite acid. The sulfur had been oxidized to sulfuric acid which had neutralized the sodium carbonate of the soil. The soil in the jar to which no sulfur was added was as alkaline as at first.

A second experiment was soon started to study the matter in more detail. The total alkalinity of a number of soils was determined by titration of a suspension in water, the theoretical amount of sulfur then added and water to bring to optimum for plant growth. From time to time the soils were tested. After 10 days it was found that from 50 to 90 per cent of the original alkalinity had been neutralized in all but two. These were very alkaline and saline at the start. Two fertile non-alkaline soils had become acid though only 0.02 per cent sulfur had been added to them (table 1). After some months the alkalinity of the alkali soils was still further reduced, but none of them became acid. The slight residual alkalinity was probably chiefly due to calcium carbonate.

For the third experiment there were taken 6.5 kgm. of an alkali soil which had been washed so that most of the soluble salts and a large portion of the alkalinity had been removed. In this soil barley germinated poorly and grew very little. A number of other plants likewise failed. Only Bermuda grass seemed to do well. The soil was dried, pulverized and mixed with the theoretical amount of sulfur to neutralize its remaining alkalinity. The sulfur used was ordinary flowers of sulfur. After two weeks most of the alkalinity had

¹ Since this article was written, the attention of the writer has been called to a note by J. G. Lipman in which the use of sulfur on alkali soil was suggested. It was dated August 24, 1916, and published in *SOIL SCIENCE*, 1916, v. 2, p. 205.

disappeared and there was a notable increase in soluble SO_4 . Twelve barley seeds were planted. A week later all the seeds had sent up sprouts, some 2 inches high. All but three were removed. These three are still growing (2 months old) and apparently fairly healthy, 10 to 15 inches high. However, their growth is much slower than would be expected in a fertile soil. The slow growth may be accounted for by the lack of available plant-food consequent to the considerable washing which the soil had received. But there was little evidence of the toxic alkalinity which had prevented the growth of barley before the treatment with sulfur.

TABLE 1
Reduction of alkalinity following additions of sulfur to alkali soil

SOIL NUMBER	SULFUR ADDED	pH	ALKALINITY OF SOIL EXPRESSED AS PER CENT H_2SO_4 NECESSARY TO NEUTRALIZE AT VARIOUS DATES				
			Beginning June 18	June 29	July 15	August 30	December 10
	<i>per cent</i>						
16	0.33	9.0+	1.00	0.70	0.70	0.54	0.30
17	0.10	9.0+	0.33	0.17	0.17	0.07	0.07
18	0.02	7.0	0.01	0.01	0.01	0.01	0.01
19	0.03	8.0	0.09	0.05	0.02	0.02	0.02
20	0.05	8.5	0.16	0.08	0.03	0.02	0.02
A	0.05	8.5	0.44	0.07	0.02	0.02	0.02
B	0.09	8.8	0.26	0.08	0.05	0.03	0.02
C	0.06	8.2	0.18	0.06	0.05	0.01	0.01
D	0.03	8.2	0.10	0.03	0.02	0.01	0.01
15	0.02	6.7	0.00	Acid			
1c	0.02	6.8	0.00	Acid			

All these soils except 1c are sandy loams. Soil 1c is a fertile clay loam; no. 15 is a fertile sandy loam.

Soils 16 and 17 have high alkalinity and high salts, no. 18 has high salts, and no alkalinity; nos. 19 and 20 have moderate salts and alkalinity. Soils A and B were produced by washing soil 17 until most of the salts were removed. Soil C is similarly derived from no. 20 and D from no. 19 by washing.

In another experiment a neutral soil was produced by mixing calculated amounts of an alkali soil with the proper amount of an acid soil which had been made by treatment with an excess of sulfur.

From these experiments it is inferred that sulfur added to a soil soon becomes sulfuric acid which reacts with and neutralizes whatever alkaline material may be present. This effect should be of great value in the reclamation of alkali land. To those who have studied the reclamation of alkali land it is common knowledge that it is very difficult to remove the last of the alkalinity by leaching. Instead of trying to remove all the alkalinity by leaching it would seem more practicable to neutralize it by the addition of sulfur after most of the salts had been removed by water. In this way the soil would not be so impoverished of available plant-food as by the long leaching which would

otherwise be necessary to remove the last of the hydrolyzable salts causing toxic alkalinity.

It seems probable that oxidation of sulfur in soil is largely due to biologic action. Assuming this to be true, it is likely that such oxidation would not take place in a soil which was too alkaline for active bacterial growth. In two such soils, 16 and 17, which will not support any ordinary plant, sulfur is very slowly oxidized, and much of the original high alkalinity yet remains. However, it would be unnecessarily expensive and usually prohibitive to neutralize all the alkalinity of such soils with sulfur. Instead, most of the alkaline matter and salts should be leached out with water, then sulfur applied to neutralize the residual alkalinity which is difficult to wash out.

THE EFFECT OF ORGANIC NITROGENOUS COMPOUNDS ON THE NITRATE-FORMING ORGANISM¹

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The behavior of the nitrifying bacteria in the presence of organic substances is a matter of special interest to the bacteriologist. It was one of the problems to which much time and energy was devoted by the Franklands, Warington, Winogradsky, Omelianski, Fremlin, Beijerinck and others. According to the results of most of these investigators soluble organic substances, especially if present in large amounts, are injurious to the nitrate-forming organism. Unfortunately the data agree neither in respect to the nature of the injury nor to the kind and amount of substances required to produce this injury. Perhaps this variation was due to the fact that in many cases the cultures under study were not pure. A review of the literature on the effect of organic substances on the nitrifying bacteria shows clearly the variation in results obtained by different investigators.

Winogradsky (8, 9) in his earlier work says that nitrifying bacteria do not require any organic substance for their growth and that even the slightest trace is toxic to them. There is every reason to suppose that the process of nitrification, which goes on in the soil in the presence of organic matter, ought not to be so sensitive to small amounts of this material in a synthetic medium. Beginning with Winogradsky, most of the investigators have reported that nitrifying bacteria do not grow in bouillon.

The Franklands (3) reported that the nitrifying organism in broth produced very characteristic growth, slow in commencing, but luxuriant; and that nitrification took place in an ammoniacal solution inoculated from such broth cultures. They found that microscopically its form differed slightly when grown in broth and in ammoniacal solutions, yet returned to its characteristic bacillo-coccus form when grown again in an ammoniacal solution. They also grew it in peptone gelatin by passing it first through broth cultures.

Warington (6) used an impure culture of the nitrous organism to seed solutions of ammonium carbonate containing 20 per cent, 5 per cent, and 1 per cent of broth. All of these solutions nitrified; they were always turbid. Stained preparations showed that the bacilli diminished in number as the proportion of broth decreased, while the deeply stained minute dots became

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more manifest. He reported that the pure nitrous organism grew slowly in weak broth, but without impairing its transparency or producing any other visible change; and that it was capable of producing nitrous acid in dilute solutions of asparagin, milk, urine and urea. All of these cultures were free from turbidity. In pure cultures of the nitrous organism in ammoniacal solutions, the addition of carbonic acid, sodium bicarbonate or calcium acetate facilitated nitrification. A pure culture would not ordinarily grow on gelatin; if it did, it lost its power of oxidation.

Fremlin (4) reported that the nitroso-bacterium would grow not only in silica jelly but also in any ordinary organic medium. He maintained that there were not two separate and distinct species of this organism; the one able to convert ammonia into nitrous acid and capable of being cultivated only in special media, the other able to grow on ordinary media with no ability to convert ammonia into nitrous acid.

Burri and Stutzer (2) reported the isolation of a nitrate-forming organism which gave a well-defined growth in the presence of organic substances which are commonly used in bacteriological culture media. Attention was called to the loss of the oxidizing power of cultures which developed in organic solutions. Examination of their culture by Winogradsky (10) showed the presence of four organisms, one of which proved to be *nitrobacter*. Since their results were obtained with an impure culture the report is not conclusive.

Winogradsky and Omelianski (11) and later Omelianski (5) alone carried out a very careful and extensive study of the influence of organic substances on the nitrate-forming bacteria. Cultures of the nitrate-forming organism from two sources, one from Germany and one from St. Petersburg, were used. Although these cultures were from widely separated sources geographically, they did not show any difference in physiological characteristics. These organisms failed to produce any turbidity in bouillon and gave a rapid oxidation of nitrite to nitrate. The following table gives the smallest concentration of the organic substance which caused a noticeable decrease in nitrate formation and the concentration which prevented it.

COMPOUND	RETARDS	PREVENTS
	<i>per cent</i>	<i>per cent</i>
Glucose.....	0.05	0.2-0.3
Peptone.....	0.80	1.25
Asparagin.....	0.05	0.5-1.0
Glycerin.....	0.05	1.00
Urea.....	0.50	1.00
Sodium acetate.....	1.50	3.00
Sodium butyrate.....	0.50	1.00
Beef broth or infusion.....	10.00	60.00

They found that the injurious effect of organic substances depended upon the composition and amount of organic substance, and also on the amount of

the inoculum. In other words, a large number of the nitrate organisms when transferred to a medium containing organic substances could withstand more organic matter than a small number. By repeated transfers to solutions containing increasing amounts of bouillon, the nitrate organism became adapted to 50 per cent of bouillon. Examination of the unoxidized cultures containing organic substances showed that the organisms were killed. In other words, failure to oxidize was due to injury of the cells rather than to growth of the nitrate ferment accompanied by a loss of its power of oxidation.

Beijerinck (1) reported the growth of pure cultures of the nitrate-forming organism in the presence of various kinds of organic substances. The chief points of interest in the paper are that growth and oxidation are two separate functions; that growth in the presence of soluble organic substances causes the nitrate-forming organism to lose its power of oxidation. He found that small amounts of the organic substance, less than 0.05 per cent of glucose, sucrose, starch, mannitol, sodium and calcium acetate, peptone, tyrosin and asparagin did not prevent the oxidation of nitrite to nitrate. He concluded that reproduction of the organisms in solutions containing appreciable amounts of organic substances caused a stable modification of their physiology. Moreover, all attempts to secure oxidation from cultures which had grown in organic solutions failed.

EXPERIMENTAL

The work reported in this paper was prompted in part by the contradictory results of previous investigators on the effect of organic substances on the growth and oxidizing power of the nitrifying organisms. It was noted in our preliminary work on nitrification that, when *nitrobacter* cultures were being tested for purity in Nährstoff-Heyden solution, contact with this organic medium did not prevent the organisms from oxidizing nitrite to nitrate when they were transferred from it to a nitrifiable medium. Because of this additional evidence, experiments were planned to include the effect of several other organic substances on the nitrate former.

For this work cultures of the nitrate-forming organisms were obtained by seeding shallow layers of liquid nitrite medium with 1-gm. portions of fresh soil. The medium was a modification of that used by Omelianski (5) and was prepared as follows:

Sodium nitrite (NaNO_2).....	1.0 gm.
Dibasic potassium phosphate (K_2HPO_4).....	0.5 gm.
Sodium chloride (NaCl).....	0.5 gm.
Sodium carbonate (Na_2CO_3) anhydrous.....	0.5 gm.
Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.3 gm.
Ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	Trace
Water distilled.....	1000.0 cc.

After 1 to 2 weeks at 28°C . the nitrites were oxidized to nitrates and subcultures were made in flasks of fresh nitrite medium. In this way the nitrate

producers were carried through 50 sub-cultures, and to the fiftieth transfer, a fresh dose of a sterilized solution of sodium nitrite (0.01 gm. NaNO_2 to 10 cc. of liquid culture) was added. This procedure of adding a dose of nitrite to the cultures as soon as they were oxidized was repeated eleven times, and these cultures are designated as enrichment cultures. The cultures were obtained from different soils; those from field soil are numbered 1, 1¹, and 1², and those from garden soil are numbered 2, 2¹, and 2².

Purity tests of enrichment cultures, in peptone-beef infusion and in Nährstoff-Heyden solution

An attempt was made to purify cultures 1, 1¹ and 1² and 2, 2¹ and 2² by subjecting them to as many oxidations of sodium nitrite as could be brought about in the same culture. At the end of each oxidation of nitrite to nitrate in these enrichment cultures, the ability of these cultures to give visible growth in either peptone-beef infusion or in Nährstoff-Heyden solution was tested by inoculating these two media with the oxidized cultures. Two tubes of each medium were used for this test, one tube being inoculated with a loopful, and one tube with 0.5 cc. of the culture to be tested.

The term "peptone-beef infusion" is used to designate a medium prepared according to the following formula: 500 gm. of fresh lean beef, 10 gm. of peptone, and water to make 1 liter. The "peptone-beef extract" consisted of beef extract (Liebig's) 3 gm., peptone 10 gm., and 1000 cc. of water. The reaction in both media was adjusted to pH 7.0 to 7.6. The Nährstoff-Heyden solution was made by heating 7.5 gm. of the Nährstoff-Heyden powder with 1 liter of distilled water for 1 to 2 hours in a steamer; the filtered solution was used without any change in its reaction, which in terms of hydrogen-ion concentration was pH 7.8. Determination of growth in these tubes was made after an incubation of 2 weeks at 28°C. The controls were uninoculated flasks of the medium, which received an addition of sterilized sodium nitrite at the same time as the cultures, and which were tested in peptone-beef infusion or in Nährstoff-Heyden solution in exactly the same manner as were the cultures. The introduction of Nährstoff-Heyden solution as a test for the purity of *nitrobacter* cultures was the result of the use of this medium for the growth of soil organisms, where it was found that it supports the growth of many more soil types than does peptone-beef infusion.

The purity tests of the enrichment cultures did not give the wholly consistent results which might be expected, namely, that growth in peptone-beef infusion would become less after each oxidation of added nitrite. But many factors might have been the cause of the irregularities with reference to growth in peptone-beef infusion: the possibilities of contamination because of the long period of time the enrichment cultures were incubated without any special protection from the dust of the air, and the possibilities of contamination when the cultures were opened for the addition of nitrite and the removal of

inocula for the purity tests, were considerable, as was shown by the fact that a number of controls became contaminated during the experiment.

However, at the end of the sixth oxidation in each of these cultures it was evident that the cultures were gradually becoming purified, since all of them gave doubtful growth in peptone-beef infusion. As compared with peptone-beef infusion a decided change was seen in the growth of these cultures in Nährstoff-Heyden solution. The seventh and eighth oxidations of the same cultures immediately following the peptone-beef infusion tests showed growth in the case of all the cultures, and this after rather doubtful growths in peptone-beef infusion. It was plainly noticeable that where all cultures showed profuse growth in Nährstoff-Heyden at the end of the seventh oxidation, at the end of the ninth oxidation, this growth was much less evident. From the results of additional experiments, it was found that after several oxidations of sodium nitrite in a culture, such a culture when inoculated into Nährstoff-Heyden solution, gave much less noticeable growth than did the same culture in which only a few oxidations had taken place.

Isolation of pure cultures of nitrobacter

In none of the tests in Nährstoff-Heyden solution did any of these enrichment cultures give negative results in both the loop and the 0.5-cc. inoculations. In addition to the usual test adopted for purity of *nitrobacter* cultures, i.e., failure to cause visible growth in alkaline peptone-beef infusion (see Wimmer (7)) it was thought desirable to apply a more critical test, and to study only such cultures as failed to give growth, after 2 weeks' incubation at 28°C., in Nährstoff-Heyden solution, when inoculated either with a loopful or 0.5 cc. of the *nitrobacter* culture.

With this test in mind, dilutions of the cultures used in the preceding experiments were made in sterilized sand water blanks. Flasks of sodium nitrite liquid medium, plates of nitrite agar, and tubes of Nährstoff-Heyden solution were inoculated from each dilution. All were incubated at 28°C. The nitrite agar was made by dissolving the salts, as given previously, in a liter of a 1.5 per cent solution of washed agar. The hydrogen-ion concentration of the resulting medium was between pH 8.8 and 9.0.

When the flasks of nitrite solution inoculated from these dilutions, after 5 weeks' incubation, were tested for the presence of nitrates and the absence of nitrites, it was found that oxidation had taken place up to and through the fourth dilution (1 in 400,000), of culture 1 from field soil, and the fifth dilution (1 in 10,000,000) of culture 2 from garden soil. Positive growth was given in Nährstoff-Heyden solution only in the tubes inoculated from the first dilution. The plates were examined after 7 weeks' incubation. The colonies in the first and second dilutions all had about the same appearance. These plates were heavily seeded with tiny white-appearing colonies; the smallest could be seen only by holding the plates up to the light and looking through them. Growth

was cut down in the third and fourth dilutions and only an occasional colony was found in plates above these dilutions.

When 8 weeks old the cultures on nitrite agar were examined again. All the plates of the lower dilutions showed numerous small colonies, cream to brown in color and often deep in the agar. Slopes of nitrite agar were inoculated from four different colonies: 1³ a brown colony which, when stained, looked like *nitrobacter*; 1⁴ a large spreading brownish colony; 1⁵ a large spreading whitish colony; and 1⁶ a small brown colony. After 17 days at 28°C. transfers were made in triplicate from these tube cultures into liquid sodium nitrite medium; and 10 days after inoculation two of the 1³ cultures were oxidized, and in 14 days a third of the 1³ cultures was oxidized. These were all transferred to sterile liquid nitrite medium and tested for purity in Nährstoff-Heyden solution; none gave visible growth in Nährstoff-Heyden solution. Cultures 1⁴, 1⁵ and 1⁶ failed to oxidize the nitrite to nitrate. In a similar manner cultures 1⁷ from field soil and 2⁸ from garden soil were secured. Here again oxidation took place and no turbidity in peptone-beef infusion or in Nährstoff-Heyden solution was noted.

Growth or existence of nitrobacter in the presence of organic nitrogenous substances

Under field conditions the presence of organic compounds of nitrogen favors in a marked degree the process of nitrification. According to the reports of many investigators, such substances are reported as having an injurious effect when added to a medium for the growth of the organisms concerned in the process. Such opposite effects hardly seem plausible and the subject deserves further study. Only a few of the possibilities along this line of investigation have been taken up in this paper.

Beef infusion. Experiments to test the toxicity of beef infusion toward *nitrobacter* were made in which 3 strains of the organism were inoculated into a fresh meat infusion (500 gm. of meat and 1000 cc. of H₂O, pH 7.6). After 2 weeks in this medium and no apparent growth, loop and 0.5-cc. portions were seeded into nitrite solutions. At regular intervals of 2 days each the nitrite solutions were tested for nitrates. Although these cultures were kept for 6 weeks no oxidation was ever noted in any of them. The data indicate the poisonous effect of beef infusion on the *nitrobacter* organism.

Peptone-beef extract and Nährstoff-Heyden. In a second test beef infusion was replaced with peptone-beef infusion and for comparison Nährstoff-Heyden solution also was used. These two nitrogenous culture solutions were seeded with *nitrobacter* cultures 1³ and 2⁸, and incubated for 14 days. No visible evidence that growth had taken place, i.e., clouding of the media, was ever noted. One-half cubic centimeter portions of these 14-day-old cultures from peptone-beef infusion and from Nährstoff-Heyden were added to flasks of the nitrite medium. The results of oxidation tests are given below:

I. From Nährstoff-Heyden:

- Culture 1⁷—One oxidized in 17 days
One oxidized in 13 days.
- Culture 2⁸—One oxidized in 20 days
One oxidized in 29 days.

II. From Peptone-Beef infusion:

- Cultures 1⁷ and 2⁸, neither oxidized.

A repetition of this experiment later confirmed the results that peptone-beef infusion is harmful and Nährstoff-Heyden, non-toxic to the *nitrobacter* organism.

In a third set of experiments, cultures 2 weeks old of different strains of *nitrobacter* 1⁸ and 1⁷ from nitrite agar slants were transferred to tubes of peptone-beef extract, Nährstoff-Heyden solution, urine, and sterilized water. Immediately after inoculation and each day thereafter, for 1 week, microscopical preparations were made from all of the cultures. These stained mounts were examined carefully, but failed to show any conclusive evidence that the organisms had reproduced. None of the tube cultures gave any evidence of clouding or turbidity. From this evidence it seems that *nitrobacter* does not reproduce in water, urine, peptone-beef extract, or Nährstoff-Heyden solution; although there is possibly a slight increase in the last-named medium.

After 12 days in these various media, transfers were made to small flasks of liquid nitrite medium. The results of these qualitative tests are given below:

1. From Nährstoff-Heyden:

- Cultures 1⁸ and 1⁷ both oxidized in 9 days.

2. From Peptone-Beef extract:

- Cultures 1⁸ and 1⁷, neither oxidized.

3. From Urine:

- Cultures 1⁸ and 1⁷, neither oxidized.

4. From Water:

- Cultures 1⁸ and 1⁷ both oxidized in 18 days.

The nitrogenous substances peptone-beef extract and urine, either killed the bacteria or destroyed their power of oxidation; while, on the other hand, Nährstoff-Heyden did not exert a harmful effect, since these cultures oxidized faster than those from water.

From the results of the preceding test it is shown that *nitrobacter* will live, perhaps reproduce, in the presence of certain organic compounds without any loss of its power to oxidize nitrites. *Nitrobacter* can be cultivated on agar which contains Nährstoff-Heyden. Experiments have been carried out to test the behavior of this organism on Nährstoff-Heyden agar slants, with and without nitrite present. It was found that pure cultures of *nitrobacter* made a fair growth on Nährstoff-Heyden agar, but a better growth when sodium nitrite was added to the Nährstoff-Heyden. When this growth on the Nährstoff-Heyden agar slants or on nitrite agar slants containing Nähr-

stoff-Heyden was transferred to liquid cultures of the nitrite medium, oxidation resulted in about 6 to 8 days. The time required for oxidation was less than that noted in the case of flasks inoculated from washed sodium nitrite agar cultures. The beneficial effect of the Nährstoff-Heyden is clearly indicated from these results.

Effect of dilution on the harmful substance found in beef infusion

To measure the degree of toxicity of fresh beef infusion, it was prepared according to the following method: 500 gm. of fresh lean beef was extracted for 4 hours at 55°C. with 1000 cc. of water. The liquid infusion was then

TABLE 1
Effect of dilution of beef infusion on the injurious factor

NUMBER	AFTER 7 DAYS IN	REACTION		
		pH	Culture No. 1 ^a	Culture No. 1 ^b
1	Sterilized water.....		All oxidized	All oxidized
2	Meat infusion.....	7.0	No oxidation	No oxidation
3	Meat infusion diluted 1-1 with water.....	6.9	All oxidized	All oxidized
4	Meat infusion diluted 1-2 with water.....	6.9	All oxidized	All oxidized
5	Meat infusion diluted 1-4 with water.....	7.0	All oxidized	All oxidized
6	Meat infusion diluted 1-10 with water.....	7.0	All oxidized	All oxidized

TABLE 2
Source of the injurious substance or substances found in beef infusion

NUMBER	AFTER 7 DAYS IN	REACTION	TIME REQUIRED FOR THE OXIDATION OF NITRITES TO NITRATES	
			Culture 1 ^a	Culture 1 ^b
			days	days
1	Sterilized water.....		7	7
2	Meat infusion sterilized for 30 minutes at 15 pounds pressure.....	7.0	No oxidation	No oxidation
3	Volatile distillate from meat infusion at 100°C.....	8.2	26	14
4	Residue from steam distillate.....	6.2	No oxidation	No oxidation
5	Ether extract of meat infusion.....	5.2	No oxidation	No oxidation
6	Residue from ether extract.....	6.5	No oxidation	20
7	Alcoholic extract of meat infusion (70 per cent alcohol).....	6.4	No oxidation	No oxidation
8	Residue from alcoholic extract.....	7.0	16	18

filtered from the beef and its reaction made to pH 7.8-8.0 after which it was boiled for $\frac{1}{2}$ hour, made up to volume, filtered and sterilized. This beef infusion was diluted with water in varying amounts and inoculated with *nitrobacter*. After 1 week, transfers were made to liquid nitrite medium. The results are given in table 1. Here it will be seen that beef infusion is

non-toxic when diluted 1 to 1 with water. The harmful substance, therefore, is present in relatively small amounts or it is only a weak poison.

Table 2 gives the results of a second experiment. If the beef infusion is subjected to a steam distillation, the harmful agent remains in the residue while the distillate is entirely free of any poison. The ether and alcoholic extracts of the beef infusion contained this harmful substance while the residues remaining after extraction with ether and alcohol were in all cases decidedly less toxic and in some cases not toxic at all. From the figures of this table it will be seen that the ether and alcoholic extracts are more acid than the beef infusion. It was thought that possibly this acid reaction was concerned with the injurious agent. New extracts were prepared and adjusted to a pH 8.0. Here again in an alkaline reaction these extracts injured the nitrate organism.

The effect of different nitrogenous organic substances on oxidation

The results from beef infusion and Nährstoff-Heyden solution with the nitrate-forming organism suggested the use of a wider variety of organic nitrogenous compounds. Accordingly, three *nitrobacter* cultures, 1³, 1⁴ and 2³, supposedly pure, i.e., no turbidity in peptone-beef infusion or Nährstoff-Heyden solution, were inoculated in duplicate in loopful and 0.5-cc. portions into the following media:

- | | |
|---|------------|
| (1) Nährstoff-Heyden 1 per cent, reaction..... | pH 7.4-8.0 |
| (2) Gelatin 1 per cent, reaction..... | pH 6.8-8.0 |
| (3) Peptone 1 per cent, reaction..... | pH 7.4-8.0 |
| (4) Casein 1 per cent, reaction..... | pH 7.4-9.0 |
| (5) Yeast water (28 gm. pressed yeast in 500 cc. of water), reaction. | pH 6.8-8.0 |
| (6) Skimmed milk, reaction..... | pH 6.4-6.8 |
| (7) Beef extract 1 per cent, reaction..... | pH 7.2-9.8 |
| (8) Sterilized water (distilled) | |

To be sure that the cultures used in this experiment contained active oxidizing organisms, transfers were made to small flasks of liquid sodium nitrite medium at the same time that the organic media were inoculated. All three cultures oxidized the nitrite completely to nitrate.

Two weeks after the various organic media had been inoculated with the *nitrobacter* cultures, flasks of nitrite media were seeded from them, 0.5-cc. portions were used as inocula. The results are given below:

1. From Nährstoff-Heyden, all cultures oxidized in 6 to 8 days.
2. From gelatin, all cultures oxidized in 6 to 9 days.
3. From peptone, cultures 1³, 1⁷ and 2³ oxidized in 8 to 12 days.
4. From casein, cultures 1³, 1⁷ and 2³ oxidized in 8 to 12 days.
5. From yeast water, cultures 1³, 1⁷ and 2³ oxidized in 12 to 15 days.
6. From skimmed milk, all cultures oxidized in 14 to 17 days.
7. From beef extract, none of the cultures oxidized.
8. From water, all cultures oxidized in 6 to 8 days.

When the same tubes of the different organic nitrogenous substances inoculated with cultures 1³, 1⁷ and 2³ were 6 weeks old, their oxidizing power was again tested in sodium-nitrite liquid medium. The results follow:

1. From Nährstoff-Heyden, all cultures oxidized in 10 days.
2. From gelatin, all cultures oxidized in 19 days.
3. From peptone, all cultures oxidized in 16 days.
4. From casein, all cultures oxidized in 12 to 14 days.
5. From yeast water, all cultures oxidized in 14 days.
6. From skimmed milk, none of the cultures oxidized.
7. From beef extract, none of the cultures oxidized.
8. From water, all cultures oxidized in 10 days.

After 2 weeks in these various nitrogenous solutions all of the cultures retained their oxidizing power except those from beef extract. In the presence of this substance the nitrate producer either loses its power to oxidize or is killed. No visible growth was noted in any of the tubes of organic nitrogenous solutions. After 6 weeks in these various media, the results were, in general, similar to those obtained after the 2-week period. The milk cultures offer an exception in that no oxidation was observed. Casein, peptone, yeast water, and gelatin retarded somewhat the oxidizing power of *nitrobacter*.

As shown from the results of these two tests, the nitrate-forming organism will live for a period of 6 weeks, perhaps much longer in a medium rich in certain nitrogenous compounds without any loss of oxidizing power. The rate of oxidation in the cultures from Nährstoff-Heyden solution was just as fast as in the cultures from water, and in certain cultures more rapid.

Effect of organic nitrogenous substances on the oxidizing power of nitrobacter in enrichment cultures

The influence of organic nitrogenous substances on the oxidation of nitrite by *nitrobacter* was studied. Here the nitrogenous substance was added direct to the nitrite medium. The three cultures, 1³, 1⁷ and 2³, which from previous tests failed to show visible growth in peptone-beef infusion or in Nährstoff-Heyden solution, were used. These nitrate formers were first inoculated into tubes of organic substances; one tube received 1 loop and another tube 0.5 cc. of the culture. After 2 weeks at 28°C. these tube cultures furnished the inocula for flasks of nitrite medium. At the time of inoculation and at the end of 2 weeks each tube culture was carefully examined by means of stained mounts, and only a few organisms were found in any of them.

The *nitrobacter* liquid cultures, the inocula of which were a 2-weeks-old suspension of *nitrobacter* organisms from one of the various organic nitrogenous substances, received, when oxidized, a second addition of 0.01 gm. of sodium nitrite. At the same time 1 cc. of a 1 per cent solution of the nitrogenous substance already present also was added to the flask cultures. The results of this second oxidation of nitrite in the presence of organic nitrogen, as well as

a third and fourth oxidation in the same culture flasks, are given in table 3. Oxidation progressed rapidly in all of the cultures except those inoculated from the beef-infusion tubes. The time required for oxidation varied according to the substance. Nährstoff-Heyden and gelatin gave the most rapid oxidation.

TABLE 3

Effect of organic nitrogenous substances on the oxidizing power of nitrobacter in enrichment cultures

NITROGENOUS SUBSTANCE ADDED	TIME REQUIRED FOR OXIDATION OF NITRITE TO NITRATE										
	Second addition of nitrite			Third addition of nitrite			FOURTH ADDITION OF NITRITE				
	4 days	5 days	7 days	3 days	5 days	12 days	2 days	3 days	4 days	6 days	10 days
<i>1cc.</i>											
Nährstoff- Heyden..	1 ^s 1 ^s 1 ⁷ 1 ⁷ 2 ^s 2 ^s			1 ^s 1 ^s 1 ⁷ 1 ⁷ 2 ^s 2 ^s			1 ⁷ 1 ⁷	1 ^s 2 ^s 2 ^s	1 ^s		
Gelatin....	1 ^s 1 ^s 1 ⁷ 1 ⁷ 2 ^s 2 ^s			1 ^s 1 ⁷ 1 ⁷ 2 ^s 2 ^s		1 ^s	1 ⁷ 1 ⁷	1 ^s 2 ^s 2 ^s		1 ⁷ 1 ⁷	
Peptone....	1 ^s 1 ⁷ 1 ⁷ 2 ^s		1 ^s 2 ^s			2 ^s					
Casein.....	1 ⁷ 1 ⁷ 2 ^s	1 ^s 1 ^s		1 ⁷ 1 ⁷	1 ^s 1 ^s 2 ^s 2 ^s			1 ⁷ 1 ⁷	1 ^s	1 ^s	
Yeast water	1 ^s 1 ^s 1 ⁷ 1 ⁷ 2 ^s	2 ^s 2 ^s		1 ^s	2 ^s			1 ^s			
Skimmed milk.....	1 ^s 1 ^s 1 ⁷ 1 ⁷ 2 ^s 2 ^s				2 ^s		2 ^s				

Effect of nitrogenous substances on the rate of nitrate formation

In this test 1 cc. of a 1 per cent aqueous solution of the various nitrogenous compounds was added at the time of inoculation to each 10 cc. of the sodium-nitrite medium. Only two cultures, 1⁷ and 2^s were used. As a control 1⁷ and 2^s were inoculated into the usual inorganic liquid medium containing sodium nitrite. The results are given below:

- (1) Nährstoff-Heyden, cultures 1⁷ and 2^s oxidized in 5 days.
- (2) Gelatin, culture 2^s oxidized in 14 days and culture 1⁷ in 18 days.
- (3) Peptone, cultures 1⁷ and 2^s oxidized in 5 days.

- (4) Casein, culture 2^s oxidized in 6 days and culture 1^r in 9 days.
- (5) Skimmed milk, culture 1^r oxidized in 5 days and culture 2^s in 7 days.
- (6) Beef extract, cultures 1^r and 2^s oxidized in 5 days.
- (7) Beef infusion, culture 1^r oxidized in 5 days and culture 2^s in 7 days.
- (8) Asparagin (0.5 per cent), culture 1^r oxidized in 14 days and culture 2^s in 18 days.
- (9) Urea (0.25 per cent), culture 2^s oxidized in 11 days and culture 1^r in 18 days.
- (10) Ammonium sulfate (0.5 per cent), cultures 1^r and 2^s oxidized in 18 days.
- (11) Controls, culture 2^s oxidized in 6 days and culture 1^r in 9 days.

If the time required for oxidation of the sodium nitrite alone is used as a control it is plain that Nährstoff-Heyden, peptone, skimmed milk, beef extract, and beef infusion favored nitrate formation. The beneficial effect of beef extract as well as beef infusion was unexpected, since in the previous test these substances were decidedly injurious. No doubt this change in effect was due to the fact that in this experiment the nitrogenous substances were present in much smaller amounts than in the former, and were mixed with the nitrite medium which would tend to overcome any toxicity of the organic compounds. A retarding effect was noted in the oxidation of cultures containing gelatin, asparagin, urea, and ammonium sulfate.

A repetition of the preceding experiment was carried out with beef extract, 1 per cent solution, and Nährstoff-Heyden, 1 per cent solution, added in varying amounts to 10-cc. liquid cultures. Strains 1^r and 1^s of *nitrobacter* which had been tested for purity by inoculation into peptone-beef infusion and Nährstoff-Heyden solution, were used. For each concentration of the nitrogenous substance 3 parallel cultures were made. As might be expected, all of these cultures did not oxidize at the same time. Below are the results of this test:

Nährstoff-Heyden:

1. Culture 1^r, in the presence of 0.5, 1.0, 2.0 and 4.0 cc. of Nährstoff-Heyden solution, oxidized in 6 to 7 days.
2. Culture 1^s, in the presence of 1.0, 2.0, 4.0, 6.0 and 8.0 cc. of Nährstoff-Heyden solution, oxidized in 5 to 10 days.

Beef Extract:

3. Culture 1^r, in the presence of 0.5 cc. of beef extract, oxidized in 6 to 8 days.
 1.0 cc. of beef extract, oxidized in 9 to 26 days.
 2.0 cc. of beef extract, only one culture, out of three oxidized in 22 days.
 4.0 cc. of beef extract, no oxidation.
4. Culture 1^s, in the presence of 0.5 cc. of beef extract, oxidized in 6 to 9 days.
 1.0 cc. of beef extract, oxidized in 6 to 9 days.
 2.0 cc. of beef extract, oxidized in 8 to 11 days.
 4.0 cc. of beef extract, no oxidation.

Controls:

5. Culture 1^r oxidized in 7 to 12 days.
6. Culture 1^s oxidized in 7 to 16 days.

Here again the favorable effect of small amounts of nitrogenous substances, especially Nährstoff-Heyden solution, on oxidation is noticeable. Beef extract also in small amounts favors oxidation. When added in greater quantities, the beef extract retards oxidation.

SOME OF THE CHARACTERISTICS OF THE NITRATE-FORMING ORGANISM USED
IN THESE EXPERIMENTS

Aside from growth in the presence of nitrogenous substances, certain characteristics of the *nitrobacter* organism were studied. Special emphasis was placed on the kind of growth on agar, the vitality of the organism, and its microscopical appearance.

Growth of nitrobacter on nitrite agar slopes

The growth of *nitrobacter* on sodium-nitrite agar slopes is very scant as compared with the growth of most organisms. It is made up of tiny beads just visible to the naked eye, more or less transparent, and of a whitish color. The culture tubes usually need to be held so that the light can come through them, in order that the faint growth along the stroke of the needle may be seen. Growth from agar slopes when inoculated into flasks of nitrite medium will cause oxidation of the nitrite comparatively fast, in a shorter time than inocula from liquid cultures.

Vitality of nitrobacter

Two strains of *nitrobacter* on agar-slope cultures which were 14 days old, were sealed and kept in the ice-box for 3 months and duplicates of these cultures were kept under the same conditions unsealed. At the end of this time, flasks of liquid nitrite medium were inoculated from them. Within 5 days one of the flasks inoculated from a sealed culture showed oxidation; in 8 days one inoculated from an unsealed tube; and in 16 days all were oxidized. At the end of a year this test was repeated and both cultures oxidized.

Three different liquid cultures of these organisms were left in the incubator at 28°C. for 30 days, at the end of which time only one drop remained of one, while two were completely dry. To see if these cultures were still alive, 10 cc. of sterilized water and a solution of 0.01 gm. of sodium nitrite were added to them. In 5 days the moist culture showed oxidation, but neither of the two which were completely dry ever gave any trace of oxidation.

Agar-slope cultures of four different strains of *nitrobacter* which had been, after the period of their incubation, kept at room temperature for 3 months and which were considerably dried, were inoculated in triplicate into liquid sodium nitrite medium. In 12 to 16 days complete oxidation had taken place in the triplicate cultures from two of the four strains, but cultures from the other two strains never gave any oxidation.

Amount of nitrate accumulated in enrichment cultures

Enrichment cultures were run of *nitrobacter* 1⁷ and 2³ to see how many additions of nitrite could be oxidized before the growth of these organisms was inhibited. The medium at the beginning contained 0.01 gm. of sodium nitrite

in 10 cc. of culture liquid. When this was oxidized, another addition of the same amount of sodium nitrite was made. In all, ten such oxidations were made covering a period of 4 months. Although sterilized water was added from time to time to make up for the evaporation in these cultures, at the end their volume was practically 5 cc. Thus the concentration was 0.123 gm. of sodium nitrate in 5 cc. of culture, or 2.46 per cent of sodium nitrate. The reaction of the cultures at this time was the same as the reaction of this medium at the beginning of the experiment, pH of 8.0 to 8.5.

Staining properties of nitrobacter

These organisms are difficult to stain and unusually so when taken from liquid cultures. The best mounts were obtained from the sodium-nitrite agar slope cultures with the use of Loeffler's flagella stain. No method of staining giving consistent results was found. Good mounts showing numerous cells were often obtained by staining freshly oxidized cultures with cold carbol fuchsin; at another time a culture of the same age and similar history would show almost no cells with this method of staining. The morphology of these organisms showed considerable variations; one of the most characteristic arrangements was that of clumps of zoöglea-like masses with only a few loose cells in the field while some mounts showed all the cells scattered more or less evenly over the field. A great many of the mounts showed the cells with rather thick straight flagella-like attachments but never more than one to a cell. Occasionally these would have the appearance of typical flagella in that they were fragile and waved. In shape the cells varied from a decided oval to almost spherical while in size equally marked differences were common.

Tubes of nitrite agar were inoculated from liquid cultures which gave no growth in bouillon or in Nährstoff-Heyden solution. After 21 days' incubation at 28°C. a faint growth could be detected. When this was stained, a small blunt bacillus, sometimes in chains, could be seen. Flasks of liquid nitrite medium were inoculated from these agar cultures and complete oxidation took place in 10 days.

Ten nitrite agar tubes were inoculated with culture 1⁷ and ten with 2³ and incubated at 28°C. Five days after inoculation a faint but noticeable growth could be seen in all the tubes. When inoculated into liquid medium all of these cultures gave oxidation, and transfers from these liquid cultures in Nährstoff-Heyden solution showed no growth. Microscopic mounts were made from the agar cultures when they were 15 days old and these were compared with mounts made from the same two cultures which were 2 months old. The organisms from the old cultures were larger and were without the flagella-like attachment, so often noted, while the 15-day cultures showed numerous polar flagella. In these mounts the flagella were twice as long as the organism and rather too thick and straight for a true flagellum. Figures 1 and 2 of plate 1, and figure 1 of plate 2 are photographs of mounts of these

cultures; and figure 2 of plate 2 is a drawing of the organisms shown in figure 1 of the same plate. The cells are unevenly stained, the center or more often one end of the cell will be well stained, while the remainder of the cell takes no stain at all except the outline of the cell wall. Often a characteristic grouping is found in these mounts similar to the illustrations.

When these cultures were a little more than 2 weeks old, they were examined microscopically again. Several good mounts were obtained. The single polar flagellum was present in all of them. Hanging-drop mounts were made from these cultures by inoculating a drop of water with growth from the agar. There was motion but it was decidedly slow. By keeping the focus carefully adjusted on one organism, it could be followed across the field; a minute or more was required for this distance.

SUMMARY

Cultures of the nitrate-forming organism were isolated from different soil types and grown on washed nitrite agar, and on slants of Nährstoff-Heyden agar with and without nitrite present. *Nitrobacter* grew on these media and retained its power of oxidizing nitrites to nitrates. Apparently the presence of a small amount of Nährstoff-Heyden was beneficial to the growth of the nitrate former. When inoculated into peptone-beef infusion or into Nährstoff-Heyden solution the pure cultures of this organism failed to show any visible growth. This statement, that no visible growth was obtained in Nährstoff-Heyden solution seemingly conflicts with the previous statement that *nitrobacter* grew on Heyden agar. Although it was not possible to determine conclusively whether growth took place in the tubes of liquid medium as shown either by its turbidity or by microscopical examinations, yet the conditions for growth in a deep layer of liquid medium compared with those on the surface of an agar slope where all the growth is concentrated in a small area, are sufficiently different to bring about results equally contradictory. Beef infusion or peptone-beef infusion, in the higher concentrations used, proved toxic for the nitrate former, whereas the Nährstoff-Heyden in nearly all tests proved non-toxic.

From examinations of microscopical preparations made from liquid cultures in water, urine, peptone-beef infusion, and Nährstoff-Heyden solution it was found that the bacteria do not reproduce in such media. Perhaps in the Nährstoff-Heyden there is a slight multiplication of the organisms.

The harmful substance found in beef infusion is present in only small amounts since dilution of the beef infusion with an equal volume of water removed this poisonous property. The nature of this harmful substance is not known, except that it is non-volatile as shown by steam distillation, and is removed by extraction with ether or alcohol.

The nitrate former will live for 2 to 6 weeks, perhaps longer, in 1 per cent solutions of Nährstoff-Heyden, gelatin, peptone, casein, yeast water, also in milk and distilled water, while in 1 per cent solutions of beef extract the bacteria are killed rapidly.

The oxidation of nitrite in cultures to which small amounts of organic nitrogen have been added takes place rapidly; in the case of Nährstoff-Heyden more rapidly than in the water controls. Such compounds as gelatin, peptone, casein, skimmed milk, beef infusion and beef extract showed no injury. Asparagin, ammonium sulfate and urea retarded oxidation.

From the results of these studies no evidence can be found to support the statements of Beijerinck that the nitrate-forming organism when grown in the presence of certain organic substances loses its power of oxidation. Contrary to much of the literature it was found that certain forms of organic matter benefit rather than injure these organisms.

Growth of *nitrobacter* on nitrite agar slants is very scant as compared with that of the common heterotrophic bacteria. The growth appears as tiny beads just visible to the naked eye. When inoculated into liquid nitrite medium, this growth brought about oxidation in a shorter time than inocula from liquid cultures.

Sealed nitrite agar slants have been kept for more than a year without serious injury to their power of oxidation.

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PLATE 1

FIG. 1. Mount made from culture 2 months old.

FIG. 2. Mount made from culture 15 days old.



FIG. 1



FIG. 2

PLATE 2

FIG. 1. *Organisms from 15-day-old culture.*

FIG. 2. *Drawing of organisms shown in figure 1.*

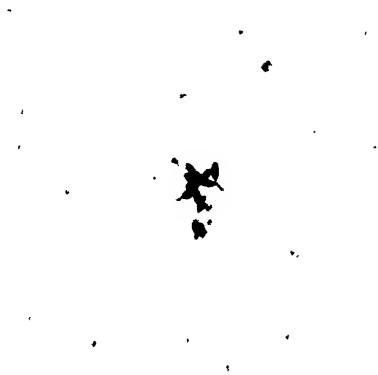


FIG. 1

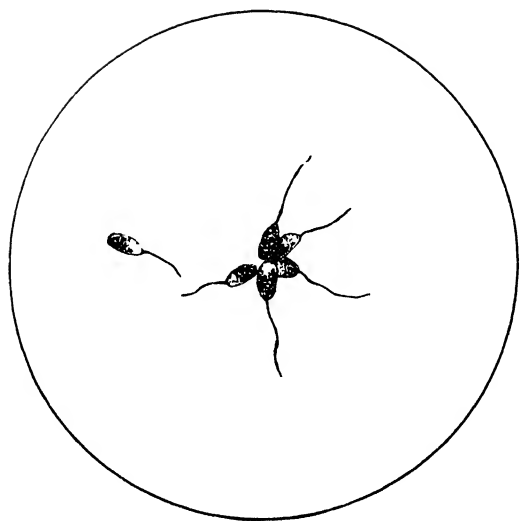


FIG. 2

AQUEOUS VAPOR PRESSURE OF SOILS

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PART I

INTRODUCTORY

A knowledge of the soil solution *in situ* is highly desirable in soil studies. This applies to the mechanical action of the soil particles upon the solution and to the influence of any dissolved material such as soil alkali. When the soil solution is removed by centrifuging or by applying pressure, it is evident that the liquid so obtained cannot be expected to exhibit the same properties as when it is distributed over the surface of the soil particles. The vastly greater water-holding capacity of clay, as compared with sand, and also the undoubted absorption of such salts as sodium carbonate present two

problems which, with their manifold corollaries, demand for their solution a comprehensive method of attack. The experimental method described in this paper, as illustrated by a few preliminary results that are herein submitted, gives promise of throwing a flood of light upon both of these problems as well as upon many other problems arising out of them.

The most obvious physico-chemical method to use in studying the soil solution directly in the soil, is the freezing-point lowering. This has been extensively employed by Bouyoucos (8) and others. There are, however, several serious objections to this method. Since the soil cannot be thoroughly stirred during the process of freezing, the observed depressions do not indicate the true freezing points and the results are therefore at best merely relative. A more serious limitation lies in the fact that the method is not applicable to the drier soils. Again, it is possible that the relations which obtain at ordinary temperatures do not hold at the freezing temperature, though these discrepancies would probably not be of large magnitude.

The dilatometer method of Bouyoucos (6, 7), should theoretically be sufficient for the solution of the moisture-energy relations of the soil—at least over a wide range. The method seems to give very significant information over a range of moisture content from somewhat less than the wilting coefficient to very wet soils, but it has thus far failed to give any detailed information about the so-called “hygroscopic” moisture except perhaps to set its maximum amount and to indicate that it is held by very powerful forces. The experimental difficulties attending the general application of the method appear rather formidable.

The conductivity method (10) may serve a useful purpose in supplying qualitative evidence as to the soluble salt content of the soil solution when the moisture content is high, but this method also fails when applied to the drier soils, and moreover only relative and empirical results can be hoped for from it.

The methods which depend upon growing plants are likewise hampered by serious limitations. Fairly accurate results can be obtained for such approximate constants as the wilting point or the toxic limits of concentrations of alkali salts when large numbers of observations are made. However, since these constants vary within appreciable limits under different atmospheric conditions and under varying bacterial conditions, it is difficult to assign any very exact theoretical significance to them.

The vapor pressure of the soil solution should supply an abundance of quantitative information about the soil. For this function, being a property of the free energy at the surface of the moisture film, is fundamentally bound up with such other properties as the osmotic pressure, freezing-point, surface tension, capillary potential, moisture equivalent, concentration of dissolved substances, and the effective diameter of the soil particle (that is, the total surface of the soil). Thermodynamic relations are known, or can be deduced, by which most of these can be calculated directly from the vapor pressure.

HISTORICAL

Highly precise measurements of the vapor pressures of certain solutions have been made by several investigators. Two general methods have been used: (1) the static and (2) the dynamic or air-saturation method.

The static method, in which the pressure differences are observed directly on manometers, was never applied to solutions with notable success until Frazer and Lovelace (15, 16, 17, 18) made it a process of great precision. Preliminary experiments in this laboratory indicated that the experimental difficulties of applying it to soils would be even greater than to solutions, and so it was temporarily abandoned in favor of the dynamic method. It is hoped, however, that the work on the static process can be resumed in the near future.

The dynamic method consists essentially in passing a known volume of air through a substance, collecting and weighing the vapor, and computing the partial pressure from the gas laws. It was first used by Regnault (29) in 1845, and it has gone through many transformations of procedure and application in the hands of Tammann (30), Walker (31), Will and Bredig (33), Orndorff and Carrell (26), Perman (28), Carveth and Fowler (13), Kahlenberg (22), Berkeley and Hartley (4, 5), Lincoln and Klein (24), Krauskopf (23), Derby, Daniels, and Gutsche (14), Washburn and Heuse (32), Baxter and Lansing (2), and others. So far as is known the dynamic method has not yet been applied to soil studies.

The greatest success in applying the method to solutions seems to have come from a suggestion of Ostwald, which was first carried out by Walker (31), that the process should be made differential by passing the same air first through the solution and then through the pure solvent, thus obtaining the relative vapor pressures of the two liquids. The apparatus was greatly improved by Kahlenberg (22) and by Berkeley and Hartley (5) who constructed saturators consisting of a series of horizontal parallel tubes connected at the ends by inverted U-tubes. The gas was passed over the surface of the liquid in these vessels while they were being rocked gently, thus causing the liquid to flow back and forth from one end to the other. Dry air was sent into the first saturator containing the solution and from there passed into a second saturator containing water. From the losses of weight of the two vessels, the vapor-pressure lowering of the solution could be readily deduced without a knowledge of the air volume. The results were very exact.

Washburn (32) made a slightly different application of the differential method. He used saturators of the same form as the above, but they were much larger and thus permitted a faster air current. Instead of weighing the saturators he absorbed the moisture from each in small absorbers containing sulfuric acid and phosphorus pentoxid. Excellent results also were obtained by this process.

A few vapor-pressure measurements of soils are recorded by Patten and Gallagher (27) who let the soil stand at 25°C. over sulfuric-acid solutions of known strength and vapor pressure, and observed the equilibrium moisture content which the soil approached from both a drier and a wetter condition.

EXPERIMENTAL

Description of apparatus

The method employed in the present experimental work is a combination of the methods of Berkeley and Washburn with some minor modifications. The apparatus is shown diagrammatically in figure 1. Its essential features consist of the following:

(1) *The constant level reservoir "A,"* from which water is siphoned under uniform head into the bottles "C" or "D" in order to force air through the apparatus.

(2) *The feed device "B,"* which serves to prevent air from collecting in the feed tubes and thus altering the rate of flow of the water.

(3) *The bottles "D" and "D₁,"* whose volumes are accurately known. The volume of the air used in an experiment can be accurately determined by adding 1 or 2 liters of water at room temperature, closing the bottle with a stopper and thermometer, and shaking until constant temperature is attained. The air is then practically saturated with water vapor at known temperature and pressure. The bottle is quickly placed in position. Knowing the time-weighted barometric pressure during the run, it is easy to calculate the total volume that passes through the apparatus. A check is thus obtained on the process by calculating the vapor pressure of water at 25°C.

(4) *The trap bottle "E."*

(5) *The loosely packed soda-lime tube "F,"* which removes any carbon dioxide.

(6) *The flowmeter "G" (3),* which consists of an oil manometer for measuring the pressure difference on the two sides of a capillary tube through which the gas is forced. This instrument must be calibrated empirically with known rates of speed, and from the curve so obtained the speed of the gas can be read directly.

(7) *The bulbs "H,"* which contain a little water over which the gas passes and becomes nearly saturated with water vapor before entering the soil tube "K."

(8) *The brass soil cylinders "K" and "K₁,"* which are 2 by 10 inches in dimensions and are connected in series by glass tubing so that the air can enter at the bottom of each one in turn. They are filled with the soil and closed by means of paraffined rubber stoppers. An oil manometer "M₂" standing in the rubber stopper attached to the second tube indicates the pressure at this point.

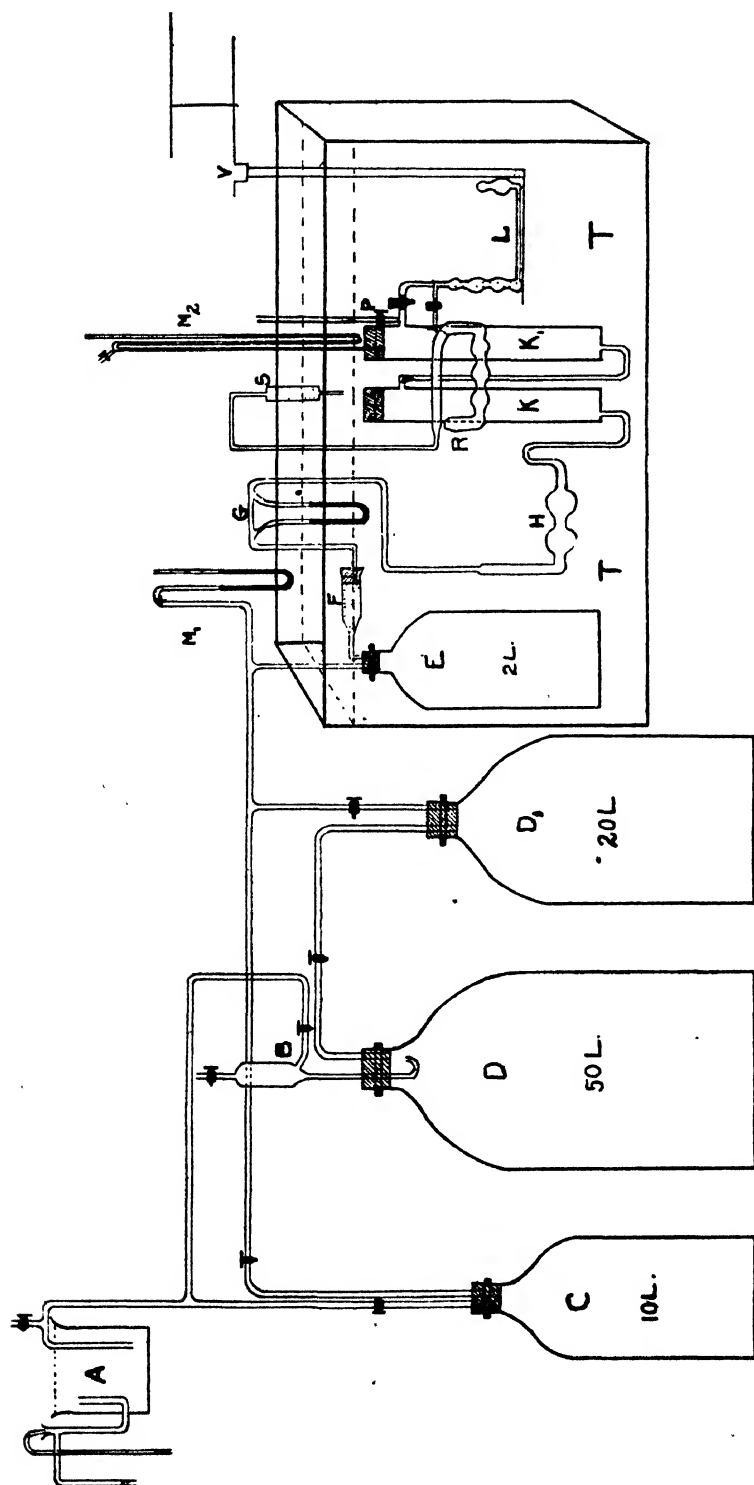


FIG. 1. DIAGRAM OF APPARATUS FOR MEASURING THE VAPOR PRESSURE OF SOILS
(See description in text)

(9) *The saturator "L."* This vessel is illustrated in figure 2. Its intake tube is fastened to the outlet of "K." It is also tied to a wire basket, the arm of which engages a rotating lever at "V" causing the outer end of "L" to be raised and lowered about four times a second. The tubes of the saturator have an internal diameter of 12 to 14 mm., and just enough water is placed in them so that the air is not at any time forced to bubble through it. The vessel is provided with two well seated glass taps. When these are lubricated with a heavy rubber grease they are unaffected by immersing in water, but it is expedient to cut off the prongs of the taps and to put two rubber caps on each of them during the run.

(10) *A glass absorber "R,"* which is illustrated in figure 3. This vessel is about 7 inches long and the two lower tubes contain about 40 gm. of sulfuric acid. The upper tube contains phosphoric anhydride on glass wool. Ten

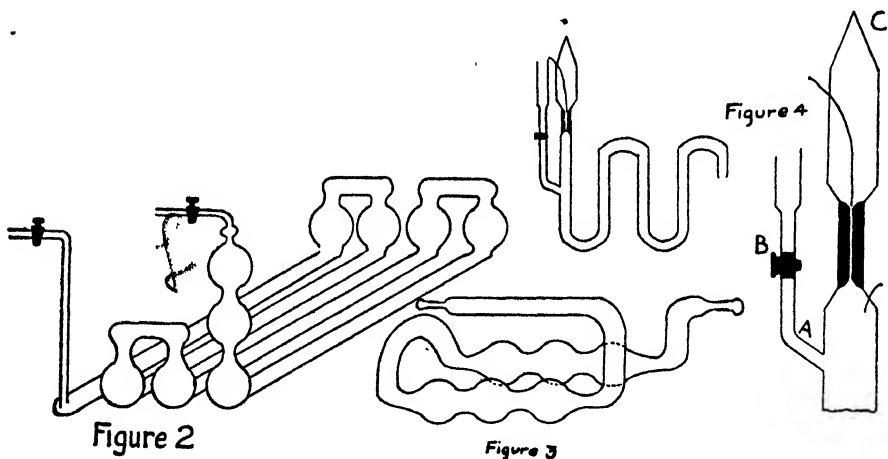


FIG. 2. THE SATURATOR

FIG. 3. THE ABSORBER

FIG. 4. THE THERMOREGULATOR

grams of water can be absorbed with this arrangement without wetting the phosphoric anhydride appreciably. The acid should perhaps be changed when 6 to 8 gm. have been absorbed. The absorber is connected to the exit tube of "L" and placed in a horizontal position. The motion of "L" serves to keep the surface of the acid agitated, thus bringing about efficient absorption. Well ground glass caps are provided for this vessel while it is being weighed.

(11) *The calcium chloride tube "S,"* which prevents back diffusion of water vapor.

(12) *The thermostat "T."* Careful temperature control is an absolute necessity in view of the fact that the temperature coefficient of the vapor pressure of water at 25°C. is about 1.3 mm. per degree, or three times the depression caused by dissolving a mole of glucose in 1,000 gm. of water. This differential method should not, however, be influenced by a slow fluctuation of temperature, provided the bath is well stirred, because the ratio of the

vapor pressure of a solution and its solvent remains practically constant over a considerable range of temperature. But in this apparatus the heat capacities of the soil cylinders and the saturator are so different that a fluctuation of temperature is disastrous.

The thermostat consists of a 30-gallon tank heated by means of electric-light globes and well stirred by a $\frac{1}{8}$ -h.p. motor. The thermo-regulator (fig. 4) is the usual mercury-toluene type, made of a thin-walled glass tube 10 mm. in diameter and about 10 feet long, bent to fit along the back of the tank. It might be well to point out one of the features of this regulator, devised by the writer, which makes it almost perfect in its operation.

The mercury end of the regulator with its two sealed-in platinum connections is shown in figure 4. The space above the capillary tube is filled with hydrogen and sealed. With this arrangement no fouling of the mercury contact has been observed after over a year of service. In filling the regulator care must be exercised to get rid of all toluene from the mercury end. This is accomplished by adding mercury to within a few centimeters below the side arm "A" (fig. 4). With the drawn-out end "C" open, air is sucked through the capillary tube until the toluene is removed. This space is partially exhausted while the tube below is heated to remove the toluene adhering to the glass under the mercury. The regulator is now filled with mercury, and hydrogen is introduced into the space above the capillary by raising and lowering the mercury, while a stream of gas is sent through a T-tube connected at "C." When the compartment is full of hydrogen the tube "C" is quickly sealed off. It is best to leave the gas under slight pressure so that when the tap "B" is opened the mercury will fall just below the top of the capillary. It is then very easy to set the instrument for operation. No variation in the temperature of the thermostat has been observed on a sensitive Beckman thermometer over long periods of time when the regulator was immersed so that the tap "B" and the platinum connection "E" were just above the surface of the water.

Preparation of the soil

Though it is quite possible that the method of preparing the soil may be a factor which influences the vapor pressure, the following process is the only one that has thus far been used in this investigation.

A large sample of air-dry soil is put through a 1-mm. sieve and thoroughly mixed. For each preparation, about a kilo of soil is mixed with the requisite amount of water or salt solution and the whole mass is forced through the same sieve twice. The material is then closed up in a 2-quart fruit jar and shaken vigorously at intervals for one or two days before use. Those soils which are wet enough to form a solid lump are shredded by forcing them through a $\frac{3}{16}$ -inch sieve. The material is placed in the brass soil tubes ("K," fig. 1) in as loose a condition as possible at the time of commencing the experi-

ment. Care should be taken to smooth the surface of the soil in the second tube, but obstructions of cotton or glass wool must not be placed in the tube leading to the saturator. Menzies (25) has shown that glass wool filters can remove appreciable amounts of water vapor from the air. With the speed of air employed in this work no trouble has been experienced in having solid particles carried over.

Weighing the saturator

The most important and the most difficult single operation in the experiment is the weighing of the saturators. An error of 1 mgm. in the weight of the saturator means an error of 0.01 mm. in the vapor pressure of the soil at 25°C. when 100 liters of air are passed. The weighing errors have been reduced to about 0.2 mgm. or less by the following method:

A balance room, maintained nearly at constant temperature and free from dust and jars, has been prepared in the center of the building. A high-class balance provided with cup-shaped pan supports is used and the balance is kept in a dust-proof box when not in use. The internal and external volumes of the saturators, and also of a sealed-up counterpoise of about the same size and shape, are known. The saturators are closed before removing from the thermostat, the internal pressure being noted, and they are not opened again during the weighing. The vessels are left in the balance for several hours before weighing them. The weights are then corrected to an average internal pressure and average balance conditions. Great accuracy is not so necessary in weighing the absorbers, but these have always been properly counterpoised and weighings corrected.

Conducting the experiment

The technique of the experiment is very simple. The saturator and the absorber are first weighed. Then the taps of the saturator are enclosed with rubber caps and the vessel is fastened to its basket. The soil cylinders are filled and connected to the saturator and absorber with rubber tubing, all rubber connections being securely wired with fine copper wire. The apparatus is put into position in the thermostat where it remains for nearly an hour in order to take the bath temperature before commencing the run. During this time the bottles "D" and "D₁" are prepared and a slow current of air from the bottle "C" is sent through the soil tubes and out through the clamp "P." This clamp is then closed, and the pressure in the apparatus is adjusted to the value which previous experiments have indicated to be necessary to send the gas through the phosphoric anhydride of the absorber (from 5 to 40 mm. of oil). The bottle "C" is now cut off and the bottles "D" and "D₁" are put into communication with the system. The pressure is again adjusted before the saturator stopcocks are opened to commence the experiment. A speed of 2 liters per hour has finally been adopted after many preliminary

experiments had indicated that erratic results are obtained when the speed is 3.5 liters per hour or greater. From 50 to 150 liters of gas are passed in an experiment. The large bottles enable the run to proceed night and day without attention, except to prevent large and sudden fluctuations in the temperature of the room. In actual practice two complete and independent sets of apparatus have been constructed so that two experiments can go on simultaneously. Enough extra saturators and absorbers also are at hand to avoid loss of time in starting a new run.

The moisture determinations have been carried out by heating 5 to 15-gm. samples of the soil to 110° to 112°C. to constant weight in a large oven into which a current of dry air was sent intermittently. The drying usually extended over several days, and even then satisfactory results were not always obtained. Indeed, an error of at least 0.05 per cent in the moisture content of the soils is probably present in most cases, due perhaps to errors in sampling and drying. This error becomes quite significant in the drier soils. Moisture determinations also were made on the soil at the top of the second tube at the end of the experiment, but no significant changes were detected. It is proposed to try to find a more accurate method of determining the moisture before continuing the work.

The soils

Four soils have been studied: (a) sand from River Heights, Logan; (b) Greenville silty clay loam; (c) clay loam from Sixth West Street, Logan; (d) a silty clay separate, the tenth soil separate prepared by Gardner (19) in his elutriator.

The mechanical analyses of these soils are given in table 1.

TABLE 1
Mechanical analyses and surface areas of the soils

SIZE		SAND		GREENVILLE LOAM		WEST LOGAN CLAY LOAM		CLAY SEPARATE	
Limits	Mean	Per cent	Area*	Per cent	Area	Per cent	Area	Per cent	Area
mm.	mm.								
1.0 -0.5	0.75	6.96	2	0.34		0.22			
0.5 -0.25	0.37	8.91	2	0.41		0.51			
0.25 -0.1	0.17	57.68	74	4.60	6	4.87	6		
0.1 -0.05	0.075	18.46	54	19.81	57	21.98	65		
0.05 -0.01	0.025	3.00	26	40.06	345	40.44	359		
0.02 -0.01	0.015							46.8	673
0.01 -0.005	0.0075	0.80	23	10.11	319	4.88	145	16.7	480
0.005 -0.001	0.0025	1.67	146	12.85	1,105	10.67	946	21.4	1,847
0.001 -0.0	0.0005	3.21	1,405	14.44	6,220	17.43	7,740		
0.001 -0.0005	0.00075							12.9	3,710
0.0005-0.0	0.00025							10.5	9,060
Total		100.69	1,732	102.62	8,052	101.00	9,261	108.3	15,770

* Square centimeter per gram.

Other physical properties are given in table 2.

TABLE 2
Some physical properties of the soils under investigation

PROPERTY	SAND	GREENVILLE LOAM	WEST LOGAN CLAY LOAM	CLAY SEPARATE
Moisture-holding capacity (Hilgard) (per cent).....	27.90	45.80	47.60	82.00
Moisture equivalent (per cent).....	3.00	23.30	24.20	35.70
Wilting coefficient (Briggs and Shantz) (per cent).....	1.39	9.30	11.00	15.00 (calc.)
Air-dry moisture content (per cent).....	0.30	2.64	2.69	4.50
Real specific gravity $\frac{25^\circ}{25^\circ}$	2.750	2.791	2.695	2.783

Complete chemical analyses of these soils have not been carried out, but their water-soluble, and carbonate contents and their organic-carbon contents as determined by the wet combustion method are given in table 3.

TABLE 3
Partial chemical analyses of the soils

	TOTAL SOLUBLE	CARBONATE (CO ₂)	ORGANIC CARBON (C)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Sand.....	0.0382	18.9	0.323
Greenville loam.....	0.1060	18.7	1.320
West Logan clay loam.....	0.1300	14.4	1.030
Clay separate.....	0.0573	15.1	0.125

In the case of the Greenville soil a few vapor-pressure determinations have been made with some soluble salts added, and in table 4 are given analyses of these soils. The water extraction was made by shaking 50 gm. of soil with 500 cc. of water for 10 minutes and filtering through a Pasteur-Chamberland filter. Only the negative ions were determined in the extracts and the results were expressed as sodium salts.

TABLE 4
Salts extracted from Greenville clay loam with various salts added

SALTS ADDED	NaCl	Na ₂ SO ₄	Na ₂ CO ₃	ADDED SALTS EXTRACTED
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
None.....	0.0120	0.0196	0.021	
0.300 per cent NaCl.....	0.310			99.4
0.729 per cent Na ₂ SO ₄		0.750		100.2
0.544 per cent Na ₂ CO ₃			0.210	36.6
1.088 per cent Na ₂ CO ₃			0.450	39.4

Results

The vapor-pressure results thus far obtained are recorded in table 5 and are plotted graphically against the moisture contents in figure 5. In their form these curves are approximately rectangular hyperbolae, as figure 5 indicates.

TABLE 5
Vapor pressure data

SOIL	SALT ADDED	DATE (1920)	SPEED OF AIR PER HOUR	WATER IN SOIL	VAPOR PRES- SURE, WATER	VAPOR PRESSURE SOIL	
						Mercury column	Mean
	<i>per cent</i>		<i>liters</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Sand.....	None	November 27	2.0	0.70	22.90*	22.737	22.737
	None	November 26	2.0	0.90	23.40*	23.332	23.332
	None	November 27	2.0	1.14	23.00*	23.491	23.491
	None	November 24	2.0	1.63	23.50*	23.579	23.579
	None	December 27	2.0	✓4.20		23.714	23.714 (?)
Greenville clay loam..	None	July 5	3.0	2.65		10.60	
	None	July 6	3.0	2.65		10.710	10.65
	None	October 22	2.0	4.45	23.80	20.628	
	None	October 24	2.0	4.45	23.80	20.537	20.582
	None	November 10	2.0	5.10	23.90	21.727	21.727
	None	August 21	2.7	6.44	23.78	22.950	
	None	August 25	2.7	6.44	23.92	22.967	22.958
	None	October 18	2.0	8.31	23.88	23.405	
	None	October 21	2.0	8.31	23.90	23.370	23.387
	None	November 10	2.0	9.52	23.88	23.470	23.470
	None	October 23	2.0	17.25	23.85	23.712	
	None	October 25	2.0	17.25		23.725	23.719
Greenville clay loam..	0.3 NaCl	July 31	4.5	4.88	23.83	20.666	
	0.3 NaCl	August 4	3.0	4.88	23.90	20.780	20.723
	0.3 NaCl	July 21	3.0	5.71	23.90	21.888	
	0.3 NaCl	July 26	4.0	5.71	23.85	21.818	21.853
	0.3 NaCl	July 29	3.0	7.12	23.80	22.890	22.890
	0.3 NaCl	December 26	2.0	7.00	23.76	22.855	22.855
	0.3 NaCl	August 5	3.0	8.87	23.76	23.165	
	0.3 NaCl	August 7	3.3	8.87	23.70	23.121	23.143
	0.3 NaCl	August 10	4.4	17.00	23.85	23.576	
	0.3 NaCl	August 12	3.9	17.00	23.90	23.506	23.529
	0.3 NaCl	August 16	3.2	17.00	23.90	23.506	
	0.3 NaCl	August 11	2.8	24.90	23.85	23.670	
	0.3 NaCl	August 13	3.0	24.90	23.85	23.570	23.603
	0.3 NaCl	August 16	3.8	24.90	23.92	23.568	

TABLE 5—Continued

SOIL	SALT ADDED	DATE (1920)	SPEED OF AIR PER HOUR	WATER IN SOIL	VAPOR PRES- SURE, WATER	VAPOR PRESSURE SOIL	
						Mercury column	Mean
	<i>per cent</i>		<i>liters</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Greenville clay loam..	0.544 Na ₂ CO ₃	August 17	2.7	5.35	23.90	21.961	
	0.544 Na ₂ CO ₃	August 19	2.7	5.35	23.87	21.988	21.974
	0.544 Na ₂ CO ₃	August 18	3.0	6.33	23.84	22.913	
	0.544 Na ₂ CO ₃	August 21	2.9	6.33	23.90	22.915	22.914
	0.544 Na ₂ CO ₃	August 23	3.4	7.96	23.90	23.198	
	0.544 Na ₂ CO ₃	August 24	3.0	7.96	23.95	23.278	23.238
	0.544 Na ₂ CO ₃	August 26	2.9	9.80	24.05	23.487	
	0.544 Na ₂ CO ₃	August 28	3.1	9.80	23.90	23.465	23.476
	0.544 Na ₂ CO ₃	August 31	3.2	15.05	23.85	23.650	
	0.544 Na ₂ CO ₃	September 3	3.1	15.05	23.90	23.661	23.656
	0.544 Na ₂ CO ₃	October 12	2.3	22.50	23.80	23.713	
	0.544 Na ₂ CO ₃	October 15	2.2	22.50	23.83	23.739	23.726
Greenville clay loam	1.088 Na ₂ CO ₃	November 2	2.0	6.77	23.90	23.009	
	1.088 Na ₂ CO ₃	November 5	2.0	6.77	23.87	22.962	22.986
	1.088 Na ₂ CO ₃	November 20	2.0	11.25	23.90	23.448	
	1.088 Na ₂ CO ₃	November 22	2.0	11.25	23.50*	23.481	23.464
Greenville clay loam..	0.729 Na ₂ SO ₄	November 18	2.0	7.39	23.90	23.067	
	0.729 Na ₂ SO ₄	November 18	2.0	7.39	23.95	23.044	23.056
	0.729 Na ₂ SO ₄	November 22	2.0	10.15	23.80	23.407	23.407
West Logan clay loam..	None	November 29	2.0	2.69		11.700	11.700
	None	December 1	2.0	5.60	23.90	22.528	22.528
	None	December 3	2.0	7.35	23.88	23.168	23.168
	None	November 29	2.0	9.55	23.85	23.490	23.490
	None	December 16	2.0	22.30		23.709	23.709 (?)
Clay separate	None	December 5	2.0	5.59		16.100	16.100
	None	December 10	2.0	8.27	23.90	22.058	22.058
	None	December 6	2.0	10.52		22.910	22.910
	None	December 20	2.0	11.30	23.85	23.011	23.011
	None	December 8	2.0	15.70	23.80	23.525	23.525
	None	December 7	2.0	29.60	24.00	23.718	23.718
	None	December 9	2.0	29.60	23.80	23.719	23.719

* A slight leak in preliminary gas train.

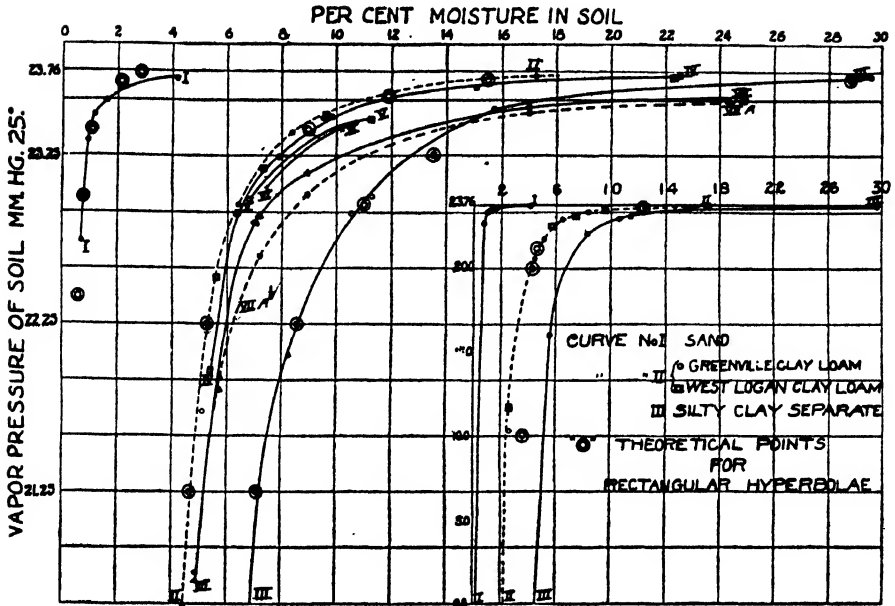


FIG. 5. DIAGRAM SHOWING VAPOR PRESSURE IN MILLIMETERS OF MERCURY AT 25°C. OF FOUR DIFFERENT SOILS CONTAINING VARIOUS QUANTITIES OF MOISTURE

Curve I sand, no salt added; Curve II Greenville clay loam and West Logan clay loam, no salt added; Curve III silty clay separate, no salt added; Curve IV Greenville clay loam plus 0.544 per cent Na_2CO_3 ; Curve V Greenville clay loam plus 1.088 per cent Na_2CO_3 ; Curve VI Greenville clay loam plus 0.729 per cent Na_2SO_4 ; Curve VII Greenville clay loam plus 0.3 per cent NaCl ; Curve VII-A, theoretical curve for Greenville clay loam plus 0.3 per cent NaCl .

The vapor pressure is thus a linear function of the reciprocal of the moisture content over a wide range. The equations representing the curves are:

$$\text{Sand: } (23.90 - p)(p - 0.35) = C_1 = 0.36$$

$$\text{Greenville loam and West Logan clay loam: } (23.96 - p)(p - 3.5) = C_2 = 3.1$$

$$\text{Clay separate: } (23.96 - p)(p - 4.8) = C_3 = 6.2$$

where p is the vapor pressure of the soil and ρ is the per cent of moisture. The significance of the constant C is of interest. It is the square of distance from the asymptotes to the point of maximum curvature and may therefore become a very important constant for characterizing the texture of the soil. The upper asymptote is very close to 23.96 in the case of the heavier soils and 23.90 in the case of the sand. This simply means that the moisture surfaces should proceed gradually from negative curvature, through the flat surface condition to positive curvature as the moisture content increases. It is doubtful if this positive curvature could be realized in practice. The curves actually cross the other asymptote in the dry soils, and according to the observations of Patten and Gallagher (27) bend off toward the origin. It is probable that the origin is not reached at zero vapor pressure, and accord-

ingly the curves have been tentatively produced to the zero ordinate. This region will be made the subject of special study later.

The positions of the curves seem to depend largely on the texture of the soil, but other factors no doubt exert minor influences. Thus the West Logan clay loam, which from the mechanical analysis, moisture equivalent, moisture-holding capacity, and wilting point must be presumed to be somewhat finer in texture than the Greenville loam, has a vapor pressure curve almost exactly coincident with the latter's. It is possible that the somewhat higher organic carbon content of the Greenville soil accounts for this coincidence.

The observed vapor pressure of water at 25°C. is included whenever possible as a check on the run. As no great effort was made in most cases to determine the exact time-weighted barometric pressure, slight variations in this value are to be expected. It was calculated from the gas laws as follows:

$$pV = NRT = \frac{WRT}{18.016}$$

where W is the weight of water absorbed, V the total volume of gas in liters that passed through the saturator and is equal to the volume of the air plus the water vapor, T the absolute temperature (298°), R the gas constant (62.4 liter-mm. of mercury), and p the partial pressure in millimeters of mercury. The formula becomes

$$p = \frac{62.4}{18.016} \frac{WT}{V} = 3.4613 \frac{WT}{V}$$

The best observed values for the vapor pressure of water at 25°C. seem to approximate 23.85 mm. As the thermostats were set at 25.08° on a thermometer graduated to tenths which gave an ice point of 0.16° and the melting point of sodium sulfate decahydrate as 32.38° (true melting point 32.384°), it is not impossible that the thermometer was correct at 25°. In that case the vapor pressure at 25° would be nearly 23.75 mm., and this value is very close to the probable value of 23.76 mm., as found by other investigators (12, 22).

Calculating vapor pressure of the soil

The vapor pressure of the soil was deduced as follows:
In the equations:

$$pV = W \frac{RT}{18.016} \tag{1}$$

$$p_o V_o = W_o \frac{RT}{18.016} \tag{2}$$

let p be the partial aqueous vapor pressure, V the total volume of the gas passing through the soil, W the weight of water in the gas in equilibrium with the soil, and p_o , V_o , and W_o the corresponding values for the same gas in the saturator.

Then dividing (1) by (2) and solving for p we get:

$$p = \frac{W}{W_o} \cdot \frac{V_o}{V} \cdot p_o$$

It is obvious that if the gas absorbs additional water vapor in the saturator V_o will be greater than V . In any case these volumes will be inversely proportional to the partial pressure of the air in them and we can write:

$$\frac{V_o}{V} = \frac{B - p}{B - p_o}$$

where B is the barometric pressure. Therefore,

$$p = \frac{W}{W_o} p_o \frac{B - p}{B - p_o}$$

Solving,

$$p = \frac{W p_o B}{B W_o + p_o (W_o - W)}$$

The value of p_o was taken as 23.760.

Good concordance was generally obtained in duplicate determinations, and it is believed that most of the later values reported are within 0.01 to 0.02 mm. of the correct values. In the case of the drier soils greater divergences are likely, but great exactness in these regions has no real significance because of errors in moisture determinations. It should be emphasized that the results in this paper are of preliminary nature only and that they are reported to illustrate the possibilities of the method rather than to give highly exact measurements. Slight changes in the apparatus were being continually made during the progress of this work, and therefore the later experiments are probably a little more accurate than the earlier ones. In its final form the apparatus gives consistent results to 0.01 mm. Still greater accuracy will very likely be possible when a contemplated improvement is embodied in the apparatus by which the slight resistance of the soil column to the air current will be reduced practically to zero.

PART II

DISCUSSION OF SOME POSSIBLE APPLICATIONS OF VAPOR PRESSURE DATA

The vapor pressure of the soil solution is fundamentally a result of the configuration and composition of the liquid phase, and two general factors may be recognized by which this function is characterized: (a) the curvature of the water wedges between the soil particles due to the surface tension of the liquid, and (b) the attractive forces of the soil mass acting upon the surface of the moisture film due to the thinness of the film. The magnitude of each of these forces will be a function of the moisture content. The influence of dissolved substances might be supposed to be a third independent variable, but since this factor can be calculated from a knowledge of the laws of solutions (if the soil is characterized for zero or a known concentration) it is obviously implicitly involved in the first two factors.

Surface tension, curvature, and vapor pressure

The relation between curvature, surface tension, and vapor pressure lowering can readily be found from a speculation involving a capillary tube of radius r standing in a liquid of surface tension t at a constant temperature T . At equilibrium the vapor pressure p at the top of the tube will be equal to the vapor pressure p_o of the flat surface below diminished by the weight of the vapor column of height h . This relation is expressed exactly by the following equation, if the vapor obeys the gas laws:

$$h = \frac{RT}{Mg} \log_e \frac{p_o}{p} \quad (1)$$

in which M is the molecular weight of the liquid and g the acceleration of gravity.

The corresponding relation between the surface tension and hydrostatic pressure is

$$h = \left(\frac{2t}{Mg} \cdot \frac{V_o}{r} \right) + (p_o - p) \frac{V_o}{Mg} \quad (2)$$

where V_o is the molar volume of the liquid.

Combining equations (1) and (2) we get:

$$\log_e \frac{p_o}{p} = \left(\frac{2t}{r} \cdot \frac{V_o}{RT} \right) + (p_o - p) \frac{V_o}{RT} \quad (3)$$

This equation states as r approaches ∞ , p approaches p_o , and as r approaches zero, p also approaches zero. Accordingly, the vapor-pressure-moisture curves should pass through the origin and perhaps be asymptotic to the

ordinate representing the vapor pressure of water. The intermediate portions of the curves will depend on the rate of change of r with the moisture content, which in turn will depend on the size of the soil particles.

The fact that the curves do not pass through the origin, but rather seem to strike the zero ordinate at appreciable moisture contents, indicates probably that as the soil becomes drier the negative curvature of the water wedges spoken of by Briggs (9, p. 19), is being augmented by the attractive forces of the soil particles acting through the thin water film to its surface. At some point on the curve the water wedges may disappear entirely and we shall be concerned only with very thin films of moisture covering the surfaces. These films may eventually become so thin that they do not exert any appreciable vapor pressure.

Capillary potential and vapor pressure

The capillary potential function " ψ " (12) is the work that must be done by the field forces in removing one mole of water from the body of the soil solution to infinity. A simple derivation involving the condensation or evaporation of 1 gm. of the vapor whose molar volume is v_o under its own pressure p_o in a large cylinder gives the relation:

$$-\psi = \frac{L_o - p_o(v_o - V_o)}{V_o} = \frac{L_o - RT}{V_o} \quad (4)$$

where L_o is the ordinary molar heat of evaporation.

If this process is now carried out at the same temperature in a capillary tube of radius r , the vapor pressure of the liquid will be lowered to p , and in order to cause evaporation from the curved surface it will be necessary to supply an amount of heat, in addition to L_o , equal to the Laplacian capillary pressure P , since the density of water is unity. The relation between them becomes:

$$-\psi = \frac{L_o - RT}{V_o} + P \quad (5)$$

but by definition

$$P = \frac{2t}{r} \quad (6)$$

and from equation (3) above

$$\frac{2t}{r} = \frac{RT}{V_o} \log_e \frac{p_o}{p} - (p_o - p) \quad (7)$$

Therefore,

$$-\psi = \frac{L_o - RT}{V_o} - \frac{RT}{V_o} \log_e \frac{p}{p_o} - (p_o - p) \quad (8)$$

The term $\log_e \frac{p}{p_o} = \log_e \left(\frac{p - p_o}{p_o} + 1 \right)$

can be expanded into a power series, and for values of p fairly close to p_o only the first term is significant, and we can write, neglecting also the term $(p_o - p)$:

$$-\psi = \frac{L_o - RT}{V_o} - \frac{RT}{V_o} \cdot \frac{p}{p_o} + \frac{RT}{V_o} \quad (9)$$

If we collect the constant terms and notice that from figure 5 the vapor-pressure-moisture curves are approximately rectangular hyperbolae and therefore

$$(a - p) = \frac{c}{(\rho - b)} \quad \angle$$

where ρ is the moisture content, we get:

$$-\psi = A + B \frac{c}{(\rho - b)} = A + \frac{D}{(\rho - b)} \quad (10)$$

where a , b , c , A , B , and D are constants.

This is the form, at least, of Gardner's potential-moisture function as deduced from Briggs' data (20, 21). It may be expected to hold over the range of moisture contents within which water can move through the soil by capillarity, for both the approximating assumptions that were made are quite valid under these conditions.

Vapor pressure and total surface of the soil

The exact experimental measurement of the total surface of a soil would be very difficult by a mechanical analysis on account of the fact that particles of lower diameter than 0.001 mm. generally contribute the largest proportion of the total surface. The equation,

$$\rightarrow \text{Total surface} = \frac{6}{D \times \text{sp. gr.}} \text{ sq. cm. per gram}$$

where D is the "effective" diameter of the soil in centimeters, indicates that the surface is inversely proportional to D . This might mean, to take an extreme example, that a sand containing 1 per cent of colloid of diameter 0.00001 mm. would have about the same total surface as a clay of a mean diameter of 0.001 mm.

One might reasonably expect, however, that the ratios of the moisture contents at zero vapor pressure, or indeed on any of the equi-vapor-pressure ordinates, along the nearly vertical parts of the curves, would approximate closely the ratios of the total surfaces of the soils. At higher moisture contents the smaller particles would very likely be completely immersed in the liquid so that the apparent total surface would be changed.

The lack of good concordance between the ratios of the moisture contents at about 10 mm. pressure, and the ratios of the calculated total surfaces is probably due, at least in part, to the insufficiency of the data given by the mechanical analysis for calculating the total surface (table 1).

Hygroscopic coefficient and vapor pressure

The explanation of the great value of the hygroscopic coefficient for characterizing soils follows at once from the above considerations. This moisture content, which represents an equilibrium condition between the vapor pressure of the soil and the partial pressure of the water vapor in the air of a room, is generally rather low down on the vapor pressure curves. It will be observed from the curves that with relative humidities from zero up to 90 per cent the range of moisture content is only from 1 to 4 per cent, according to the texture. Therefore, if care is taken to control the relative humidity, to say 10 per cent, the moisture content would be accurate to 0.1 to 0.4 per cent. It should be emphasized, however, that the term "hygroscopic coefficient" has no real meaning unless the relative humidity is specified. For if a soil were allowed to stand long enough in a completely saturated atmosphere it would go on taking up moisture until the water wedges attained zero curvature. This would probably result in a very wet soil. The experiments of Patten and Gallagher (27) seem to bear out this conclusion, though they emphasize the fact that equilibrium is attained very slowly. More rigid temperature control than 0.1° is necessary to give the experiments finality. It is not unlikely, however, that every soil is characterized by more than one vapor pressure-moisture curve according to the mode of preparation, in which case the above conclusion would have only limited application. Experiments are now being planned to investigate this matter.

The wilting coefficient and vapor pressure

The values of this soil constant, as determined by the method of Briggs and Shantz (6) are given in table 2 for three of the four soils. Tepary beans were grown. The numbers given are the averages of 75 tumbler determinations. The wilting point for wheat also was determined on Greenville soil. The clay separate was not investigated, but its wilting coefficient has been calculated from the moisture equivalent by dividing by 2.4, which was the ratio for the other soils. It will be seen from table 2 and figure 5 that the

plants invariably wilted when the vapor pressure of the soil commenced to fall rapidly. The vapor-pressure depressions corresponding to these moisture contents were as follows:

(1) Sand (beans).....	$W = 1.39$ per cent H_2O	$(p_o - p) = 0.20$ mm.
(2) Greenville loam (beans).	$W = 9.30$ per cent H_2O	$(p_o - p) = 0.30$ mm.
(3) Greenville loam (wheat)	$W = 7.70$ per cent H_2O	$(p_o - p) = 0.46$ mm.
(4) West Logan clay loam (bean).....	$W = 11.00$ per cent H_2O	$(p_o - p) = 0.20$ mm.
(5) Clay separate.....	$W = 15.00$ per cent H_2O (calc.)	$(p_o - p) = 0.30$ mm.

It is evident from the above discussion that the wilting point for each soil can be characterized fairly definitely from its vapor-pressure curve. Naturally, in the direct determination of this value the atmospheric temperature and humidity will play important rôles and should be controlled. A very serious error results from the difficulty of judging when the plants are wilted. The inconsistencies in the literature in the wilting-point determinations are no doubt assignable to these causes. The function might be more accurately defined in terms of a definite vapor-pressure depression. This would mean that plant roots would have to exert a definite arbitrary force at this point to take up the water from the soil, whether the plant leaves were transpiring the moisture at the same rate as the intake at the roots or not. It might be mentioned that a vapor-pressure depression of 0.4 mm. in a solution at 25° means an osmotic pressure of about 26 atmospheres.

"Unfree" water and vapor pressure

In determining the "unfree" water in a soil Bouyoucos (6) measures the amount of water that does not freeze at -4° . When equilibrium is reached the moisture remaining on the soil will have the same vapor pressure as the ice at -4° . Assuming von Babo's law to hold, one finds from the data of Frazer, Lovelace, and Sease (18) that this condition would correspond to a vapor-pressure lowering at 25° of 0.91 mm. Bouyoucos states¹ that his "unfree" water content is only slightly lower than the wilting coefficient for wheat, the value of which for the Greenville soil has been found to be 6.9, 8.0, and 8.2 per cent, respectively, with a mean of 7.7 per cent. These values were determined at different times during the summer of 1920, each number being the mean of 25 experiments. The discrepancies are due largely to the difficulty of deciding when the wheat was wilted. A vapor-pressure depression of 0.91 mm. corresponds to 6.4 per cent moisture in this soil.

¹In a later paper Bouyoucos states that the wilting coefficient corresponds more nearly with the -1.5° reading of his dilatometer than the -4° reading. This is in much better agreement with the results recorded above because a freezing-point depression of 1.5° would correspond with a vapor-pressure depression at 25° of 0.338 mm.

Moisture equivalent and vapor pressure

A complete solution of this problem is impossible at the present time on account of the lack of sufficient experimental evidence. It will be seen from figure 5, however, that the moisture equivalent corresponds to a vapor-pressure depression of about 0.02 to 0.04 mm. Unfortunately, on this portion of the curves the vapor pressure changes are very slight for large variations of the moisture content so that exact characterization of the curves can be obtained only when the precision of the vapor-pressure measurements reaches 0.001 mm.

A partial solution of the problem is outlined below. Since the centrifugal force of the machine tending to throw the water out of the soil is exactly counterbalanced at equilibrium by a negative moisture gradient, the observed "moisture equivalent" will decrease as the thickness of the soil layer in the centrifuge cup increases. Table 6 gives some observed values of the moisture

TABLE 6

*"Moisture equivalents" of Greenville soil when varying amounts of soil were placed in cups
(Radius of centrifuge 153 mm.)*

SOIL	THICKNESS OF LAYER	WATER		
		1	2	Mean
gm.	mm.	per cent	per cent	per cent
5	1.7	32.8	30.6	31.7
10	2.8	29.5	28.8	29.1
20	5.5	24.9	25.0	24.9
30	8.2	23.0	22.6	22.8
40	10.8	21.0	20.8	20.9
50	13.5	19.4	19.8	19.6
60	16.2	19.9	19.1	19.5
70	19.0	18.5	19.1	18.8

contents for the Greenville soil when varying amounts of soil were placed in the cups and centrifuged at 2460 revolutions per minute for 45 minutes.

The moisture determinations were made by drying the whole sample and therefore represent the values for the center of the blocks.

A correlation between these data and the vapor pressure can be found by making use of the potential function when it is remembered that the centrifugal force can be equated to the potential gradient:

$$\frac{d\psi}{dr} = r\omega^2 \quad (11)$$

where r is the radius of the machine to the element of the soil under consideration and ω is the angular velocity of the machine. On integration we get:

$$\psi = \frac{r^2\omega^2}{2} - \psi_c \quad (12)$$

where ψ_0 is the value of the potential at the center of the centrifuge. This has not been determined, but the equation can be used with the data in table 6 by taking two values of r :

$$\psi_1 - \psi_2 = \frac{\omega^2}{2} (r_1^2 - r_2^2) \quad (13)$$

This equation can now be combined with equation (9) to give:

$$\psi_1 - \psi_2 = \frac{\omega^2}{2} (r_1^2 - r_2^2) = \frac{RT}{V_0} \frac{(p_1 - p_2)}{(p_0)} \quad (14)$$

From the above data the 70-gm. block of soil would contain 31.7 per cent of water in the outside 5-gm. layer, about 18.5 per cent at the center, and about 16 per cent in the inside 5-gm. layer. The calculated vapor-pressure depression from the outside to the center is 0.0148 mm. and from the outside to the inside 0.0288 mm.

The observed values in this region, though not determined in an altogether satisfactory manner, are from the slope of the curves, of this order of magnitude. Since the centrifugal force is 1000 times gravity it is interesting to observe that the vapor-pressure change in the centrifuge, as determined above, agrees closely with the change as calculated from a vertical column of water vapor, 1000 times the height of the soil column. The change of pressure in saturated water vapor at 25° is 0.00171 mm. per meter of elevation. A layer of soil in the centrifuge, 17.4 mm. thick, should give a vapor-pressure range of 0.0288 mm., while the corresponding vertical column, 17.4 meters high, should give a range of 0.0298 mm.

The influence of dissolved materials on vapor pressure of the soil

A study of this phase of the problem was originally the main purpose of this investigation. The concentration of the dissolved material is related to the vapor-pressure lowering by the well-known Raoult law:

$$\frac{p_0 - p}{p_0} = \frac{N}{N_0 + N}$$

where N represents the number of moles of solute in N_0 moles of solvent. This relation is only applicable as it stands to "ideal" solutions and to dilute solutions of non-electrolytes. In the case of aqueous solutions of electrolytes approximate agreement is found by using the law, if instead of N we employ

$$(1 + ni)N$$

where n is the number of ions formed from one molecule of N and i the degree of ionization as found by the conductivity method. It has been shown, however, by Frazer and Lovelace (16, 18) that the molar depression of the vapor

pressure of potassium chloride is practically constant over the range 2.0M to 0.4M in spite of the fact that the conductivity indicates appreciable change in the amount of dissociation over this range. We are dealing, therefore, in the case of the soluble salts with complex thermodynamic relations to which Raoult's law and the other "laws of solution" do not apply rigorously, and for the complete solution of the problem from this point of view it will be necessary to measure the vapor-pressure depressions of the pure solutions of all the salts studied.

All of the thermodynamic properties of a solution are connected, however, by a set of equations into which the concentration of the solute does not enter. These "colligative properties" enable us to connect the freezing-point lowering, the osmotic pressure, the surface tension, the vapor-pressure lowering, etc. in a very satisfactory manner. Therefore, in the absence of vapor-pressure data for the common "alkali" salts we can make use of freezing-point data for purposes of comparing the observed depression of the vapor pressures of the soil with the depressions in the corresponding simple solutions.

The curves VII, IV, and VI in figure 5 illustrate the results that have thus far been obtained by adding equimolar amounts of sodium chloride, sodium carbonate, and sodium sulfate (0.3, 0.544, and 0.729 per cent, respectively, of the dry soil) to the Greenville soil. It will be noticed that the concentration of the soil solution should increase as the soil becomes drier.

The theoretical curve for sodium chloride (curve VII A) has been prepared from the data of Frazer, Lovelace, and Miller (16, 18) on the assumption that the vapor-pressure effects of the two salts are identical. This assumption seems justifiable because the molar freezing-point lowerings of the two salts are practically identical (Landolt-Börnstein tables). The agreement of the theoretical and experimental curves seems rather remarkable and indicates that there is only slight absorption of this salt, except at the region of maximum curvature, where the absorption increases to about 44 per cent.

The theoretical values for 0.544 per cent sodium carbonate have likewise been deduced from freezing-point data, but they are not shown in figure 5. They would be represented by depressions about 18 per cent greater than those shown in the theoretical curve VII, A. From this calculation it appears that the carbonate is in some way removed from the soil solution to the extent of 85 to 90 per cent. The absorption seems to increase as the soil becomes drier so that the curves approach rather than recede from each other as the moisture content decreases. The theoretical depressions for 0.729 per cent sodium sulfate would correspond closely with those given by sodium carbonate. The absorption indicated by this calculation therefore is about 70 per cent. The data are insufficient, however, to warrant the drawing of further conclusions at this time, but they indicate that it will be possible to secure much valuable information in regard to the concentration of the soil solution *in situ*, by further vapor-pressure studies.

SUMMARY

Part I

1. A method of measuring the aqueous vapor-pressure lowerings of soil, accurate to 0.01 mm. of mercury at 25°, is described and a few preliminary experimental results are given illustrating four different soils as well as the influence of adding the common "alkali" salts to one of them.

2. The vapor-pressure-moisture curves are shown to be rectangular hyperbolae over a wide range of moisture contents. This means that the vapor pressure is proportional to the reciprocal of the moisture content.

3. The position of each curve depends almost wholly on the texture of the soil in the absence of dissolved material.

Part II

4. Sodium carbonate, sodium sulfate, and sodium chloride are shown to be absorbed by the Greenville soil to the extent of about 85 to 90, 70, and 10 to 44 per cent, respectively, though the last two salts can be extracted completely.

5. Some of the energetic functions of these soils are analyzed in the light of vapor-pressure data and their significance pointed out from this viewpoint. Correlations are thus given between the vapor pressure and the following properties: hygroscopic coefficient, wilting coefficient, moisture equivalent, Bouyoucos' "solid water," capillary potential, surface tension, and curvature of the moisture surface. In the case of the moisture equivalent some independent data and calculations are given confirming a portion of the vapor-pressure data.

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The present work is a natural outgrowth of researches which have been going on for a long time in this laboratory under the direction of Dr. F. S. Harris on moisture, soil, crop and alkali relations. The author takes this opportunity of thanking Dr. Harris for continued assistance and encouragement during the progress of this work. He also desires to thank Dr. Willard Gardner for helpful criticism of the theoretical discussion in this paper. The mechanical analyses were painstakingly carried out by Dr. D. S. Jennings and Mr. H. E. Flanders. Messrs. Marion L. Harris, Karl Harris, Granville Oleson, Leslie Jenkins, and D. W. Pittman, and Mrs. Lilaine O. Thomas have assisted at various times with the experimental work.

The work is being continued, but it is hoped that the results from this method of attack will appeal to other investigators as being worth the labor involved and that vapor-pressure studies will take an important place in soil researches.

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THE INFLUENCE OF CERTAIN FERTILIZER SALTS ON THE GROWTH AND NITROGEN-CONTENT OF SOME LEGUMES

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INTRODUCTION

Since the time of Hellriegel and Wilfarth (1888-90) who established the fact that symbiotic bacteria are responsible for nitrogen-assimilation by legumes, and that of Beyerinck (1888), who isolated the specific causal organism (*Bacillus radicicola*), the scientific world has believed that leguminous plants obtain the bulk of their nitrogen from the atmosphere. In recent years it has been fully demonstrated by a number of investigators also that calcium plays an important part in the soil in increasing the activity of this symbiotic organism and hence in stimulating the assimilation of nitrogen by legumes. It has, however, not been so fully shown just what fertilizing elements, other than calcium, and what combination or combinations of these elements best promote this nitrogen-assimilation and legume growth generally. To shed further light, if possible, on this somewhat obscure topic the investigation herein described was undertaken. In addition to the work of ascertaining the effect of certain fertilizer salts, containing the elements that it was thought fit to study specially, on the growth (dry-matter) and nitrogen-content of a few legumes, it was deemed advisable to investigate also the effect of these salts and of the resulting crop growth on subsequent soil nitrification.

. HISTORICAL

It has been stated in the introduction to this thesis that in recent years it has been fully demonstrated that calcium plays an important part in the soil in stimulating symbiotic organisms and hence in promoting the growth of most legumes. Thus it was deemed advisable not to include lime in this investigation as a subject for further study but to provide each treatment, including that for the checks, with calcium carbonate. A mere reference here to the investigators of recent years who have found lime in various ways beneficial to legumes therefore must suffice. The list includes the following, in order according to the date of their published writings on the subject: R. Ulbricht (57); C. G. Hopkins (23); A. F. Khandurin (25); D. N. Prianisch-

nikov (46); T. L. Lyon and J. A. Bizzell (40); J. F. Duggar and M. J. Funchess (7); J. B. Abbott (1); J. G. Lipman, A. M. Blair, I. L. Owen, and H. C. McLean (36); Lipman, Blair, McLean and Wilkins, L. K. (37); Lipman and Blair (30, 31, 32, 33, 34, 35); W. Frear (14); F. W. Morse (43); E. B. Fred and E. J. Graul (15, 16); H. W. Truesdell (56); J. K. Wilson (60); C. R. Fellers (9). Doubtless there are other investigators who may be cited, but the experimental findings of the above-mentioned are adequate for purposes of establishing the fact that lime is beneficial in various ways to legumes as a whole.

The literature on the subject of the effect of various nutrient salts, other than calcium, on the growth and nitrogen-content of legumes, is not as extensive as that associated with lime and its effects thereon. Nevertheless, a search revealed a fair supply of published matter, particularly with reference to the action of individual elements, such as phosphorus and sulfur, on certain phases of the growth of legumes. This literature is cited below in chronological order within the citation of the published material in general appertaining to experiments with a particular nutrient element.

On the subject of the effect of nitrogen, in various forms, on the assimilation of atmospheric nitrogen and on the growth thereby of legumes, the following citations are furnished:

1910. Löhnis (39) discussed at length the earlier history of the investigations associated with this question. He cited numerous investigators and stated their individual contributions to this controversial topic, listing among the supporters of the idea of nitrogen-fixation by legumes in the presence of abundance of nitrogen, both organic and inorganic, Prazmowski, Beijerinck, Frank, Böhme, Aeby, Bäsler, Nobbe and Hiltner. On the other hand there were cited the names of investigators whose work in general favored the idea of non-fixation in the presence of strong nitrates, of ammonium nitrates or sulfates, of strong accumulation of nitrogen from continuous manuring, or of water cultures. These workers included Wohltmann and Bergené, Vines, Laurent, Nobbe and Richter, Mazé, Marchall, Flamand, and Hiltner.

1914. Lipman and others (30), by means of pot experiments, showed that there was little difference in the yield and nitrogen content of soybeans fertilized with varying quantities of acid phosphate, nitrate of soda, gypsum and calcium carbonate. Gypsum gave the lowest percentage of nitrogen. Calcium carbonate, nitrate of soda, or acid phosphate in double quantity did not affect the protein content of the plant appreciably, although this increased the yield.

1915 and 1916. Lipman and Blair (31, 32, 33, 34) found that the nitrogen content of soybeans increased with applications of nitrate of soda, ammonium sulfate and dried blood. They also found that in sand cultures nodule development was not depressed by nitrogenous fertilizers, and that therewith the yield of dry matter increased up to a maximum and then decreased.

1916. Shive (52) found salts, except in weak concentrations, injurious to soybeans grown in sand. Ammonium salts, other than ammonium sulfate, exerted a more toxic action on soybeans than any of the corresponding salts of potassium, sodium and calcium.

1917. J. K. Wilson (60) pointed out the effect of various salts on nodule development. In general, chlorides, phosphates, calcium compounds and carbon-containing compounds seemed to stimulate nodule formation, while sulfates and ammonia-containing fertilizers depressed this formation on soybeans.

1917. Truesdell (56) found that the use of nitrogen did not increase the number of nodules on alfalfa roots. Nitrogen had apparently a depressing influence on the air-dry weights of the first cutting of this crop, grown in uninoculated soil, but it had no harmful effects on subsequent cuttings. He also found that the addition of nitrogen to the soil increased the total nitrogen in the roots of alfalfa.

1918. Fellers (9) showed that nitrate of soda increased the yield of soybeans but inhibited nodule formation and consequent fixation of atmospheric nitrogen, and concluded that it is not economical to supply soluble plant-food in the form of nitrogenous fertilizers to this crop. Nitrate of soda caused an appreciable increase in the protein-content of soybean seeds.

1918. Hills (22) found that the presence of large amounts of potassium, sodium, and calcium nitrates proved detrimental to the formation of nodules on alfalfa. Alfalfa seedlings grown in the presence of large amounts of nitrate did not produce nodules when inoculated with a viable culture of *B. radicicola*. Nitrates in soil cultures prevented the re-formation of nodules once removed and also caused a decrease in the number of nodules already present.

1920. Albrecht (2) concluded from his investigations that nitrogen fixation will take place in a soil containing large amounts of nitrogen in the form of either nitrates or organic matter, that no injurious effects on nitrogen fixation are caused by nitrates, that nodules are produced in the presence of large amounts of organic matter, and that variations in total nitrogen of a soil fail to affect nitrogen fixation.

On the effect of phosphorus upon the legume phenomenon, the following citations may be made:

1916. Shive (52), growing soybeans in solutions, found that phosphates caused injury to most of the seedlings where high concentrations of the radical PO_4 were employed.

1917. Truesdell (56) concluded that a part of the benefit to higher plants from phosphorus was due to some additional factor other than cellular stimulation and the quickening of soil bacterial processes, as suggested previously by Fred and Hart (17) and Lipman (29).

Working with Miami silt loam in earthenware jars under greenhouse conditions, Truesdell grew alfalfa with phosphorus (dicalcium phosphate) and phosphorus plus nitrogen (urea). The beneficial effect of phosphorus on

plant growth was noted almost from the start, and this rapid early growth may be accounted for, according to the author, only as a result of direct nutrition and stimulation of the plant by phosphorus, and as a result of the quickening of bacterial actions other than those connected with nitrogen fixation.

Phosphorus increased the formation of nodules, and this finding substantiated the previous investigations of Marchal (4), Laurent (26), Wohltmann and Bergené (61), Löhnis (34), Deherain and Demoussy (6), Flamand (10), Prucha (47), and J. K. Wilson (59).

Analyses of the roots of alfalfa showed an increase in the total nitrogen-content due to the addition of phosphorus.

The percentage of nitrogen in the first alfalfa cutting varied in inverse proportion with the dry-weights, this being in agreement with numerous observations that rapid-growing plants contain a smaller percentage of nitrogen, on dry-weight basis, than slow-growing plants. But here, though the phosphorus-treated plants grew faster than the controls, yet the total nitrogen was greater in this phosphorus-treated alfalfa.

Analyses of the third cutting, which was deemed more representative of the normal mature growth of the crop, showed an entire agreement between the results, due to phosphorus, obtained from the whole crop and from the first cutting, by way of increased total nitrogen and increased dry weight. But the third cutting showed an increase in the percentage of nitrogen in the tops for phosphorus treatment. Phosphorus caused a greater total of nitrogen and a greater percentage of nitrogen to be stored in the tops than did nitrogen treatment of the soil. The data for the inoculated and the uninoculated series agreed throughout. Consequently, the author concluded that the difference in the percentage of nitrogen must unquestionably be considered as resulting from phosphorus treatment. The results obtained by the use of phosphorus were (a) increased growth, and (b) greater efficiency in fixing and storing nitrogen. The nodule bacteria apparently had not only supplied more nitrogen to those plants that received heavier treatments of phosphorus, but had also stored a larger percentage of nitrogen in their tops.

The data seemed to indicate increased activity of root bacteria due to phosphorus, resulting in the above-mentioned benefits. This relation was especially evident in the third cutting where an additional benefit from phosphorus was expressed in the occurrence of an increased percentage of nitrogen.

1918. Fellers (9) concluded from field experiments with soybeans that the yields of total dry matter and seed are materially increased by small applications of acid phosphate, especially on well limed soils. One to two hundred pounds appeared to be as beneficial as large applications. He also found that nodule formation on soybeans was stimulated, on limed soils, by acid phosphate. The stimulation was not so marked on acid soils. This fertilizer seemed to exert a beneficial influence on protein formation in the seed on both limed and unlimed plots. The fertilizer treatment for soybeans that appeared to give the best return for the money invested was probably 200 to 400 pounds of acid phosphate, together with a ton of lime, per acre.

The intimate relation of potash to nitrogen-assimilation by legumes has in the past been definitely established by various investigators. Recent investigations on the subject of potash fertilizing of legumes however may be cited.

1918. Fellers (9), by field experiments, showed that muriate of potash in applications of 50 to 400 pounds per acre gave an average increase of about 10 per cent in the yield of total dry matter and seed of soybeans on both limed and unlimed plots. Nodule production was slightly stimulated on the limed plots but not on the unlimed. Potash, he found, had little influence on the protein content of the seeds of soybeans.

The literature on the subject of the effects of sulfur upon the growth and nitrogen content of legumes is fairly extensive, and from this published material the following citations may be made:

1911. Hart and Peterson (20) called attention to the apparent deficiency of sulfur in certain soils as related to the demands made upon this element by some species of agricultural plants, legumes included. Analyses of these crops showed alfalfa especially high in sulfur content, and that this crop's sulfur requirements were actually greater than the phosphorus requirements.

1912. Bernard (3) found crop increases from the use of sulfur.

1912. Boullanger (4) obtained increased yields of crops from the sulfur treatment of the soil.

1913. C. B. Lipman (27) concluded that gypsum stimulated the beneficial soil organisms on the roots of legumes.

1914. Lipman and Blair (30) fertilized soybeans grown in pots, with calcium sulfate, nitrate of soda and calcium carbonate at applications of 10 gm. and 25 gm. The maximum yield of soybeans for a single pot was obtained from the calcium sulfate treatment.

1914. Shedd (50) obtained beneficial effects from sulfate with various crops grown in soil cultures. There were decided gains in the growth of soybeans with applications of sulfur, ammonium sulfate, pyrite and ferrous sulfate and smaller gains with calcium, potassium, barium, magnesium, aluminum and sodium sulfates on a soil containing 600 pounds of sulfur and 3040 pounds of phosphorus per acre.

1914. Reimer (48) obtained increased yields of alfalfa grown in the presence of flowers of sulfur.

1915. Hart and Tottingham (21), by means of soil cultures in the greenhouse, found that sulfur in the form of calcium sulfate, more so than in the form of sodium sulfate, was beneficial to common red clover, especially lengthening its root-system, hence feeding power, and increasing the yield of the dry matter 23 per cent. They showed also increased yields of legumes with calcium sulfate added to a complete fertilizer over a complete fertilizer plus potassium chloride. Here, they claimed, the action of the calcium sulfate must have been direct.

The same investigators found that calcium sulfate was especially favorable in increasing the yield of grain in peas. Its effect in increasing straw was more in evidence with beans and red clover.

1916. Pitz (45) concluded that calcium sulfate in small amounts increased the yield of red clover and the formation of nodules. Sulfates stimulated the development of red clover bacteria as well as the young plant. Elemental sulfur, however, increased the yield of red clover but slightly, and did not affect the root development nor the formation of nodules.

1916. Duley (8) found that when used alone on silt loam soil, flowers of sulfur was beneficial to the yield of red clover. It also very markedly increased nodule production on the roots of red clover when added to a complete fertilizer.

1917. Shedd (51) grew soybeans, red clover, alfalfa, and other legumes with 100 to 200 pounds of flowers of sulfur. He found that in the soybeans, which showed an increased sulfur content, no corresponding increased protein content always was found. In five out of eight instances, however, soybeans grown in soil where sulfur was added showed an increase in the total weight of protein.

1917. Brown (5), from experiments conducted in the Hood River Valley of southern Oregon, states that sulfur is a valuable fertilizer for alfalfa, the sulfur content of which is very high, according to the experiment station analyses. There air-slacked lime failed to produce increased yields of alfalfa, but when followed by a 100-pound application of land plaster (calcium sulfate) at the end of the first cutting, the plants immediately took on renewed vigor and easily surpassed the unfertilized plot on a total season's yield by the end of the last, or third cutting. This increase was shown despite the fact that the first cutting showed 1168 pounds for the check versus only 480 pounds for the other. The experiments with flowers of sulfur did not show such large increases of alfalfa, and it would seem, stated the author, that the lighter applications are the most economical when applied each year. Sulfur being quite insoluble in water, hence not immediately available, it was recommended that it be applied in the fall or not later than January or February, whereas land plaster should be applied as early as March to produce good results.

1918. Tottingham (55) showed that the addition of sodium sulfate and calcium sulfate to the sulfur-free modification of Knop's solution, in amounts equivalent to the sulfur of the unmodified solution, produced a greater yield of dry tops of red clover than did the latter solution, calcium sulfate being very efficient in this respect. It appeared as if the sulfur of gypsum functioned in the molecular combination in which it was supplied. The data obtained indicated that a deficiency of sulfur supply restricts growth by limiting the synthesis of protein. The author stated that the more or less parallel fluctuations of the plane of sulfur supply, the weight of nitrogen assimilated, and the yield of dry tops of the red clover plants, indicated that sulfur deficiency restricted growth by limiting this synthesis of protein.

1919. Miller (42) concluded that the great increase found in the nitrogen content of the clover grown in soil where sulfate had been added, is the result, in all probability, of these sulfates stimulating the action of legume bacteria.

His experiments also showed that sulfates caused an increase in root development and in the number of nodules on the red clover roots.

1919. Reimer and Tartar (49) found that on various types of soil alfalfa and red clover were increased from 50 to 1000 per cent by the use of various types of fertilizers containing sulfur, gypsum included. The soils ranged from coarse granite soils to the heaviest adobes. None were acid nor noticeably alkaline. Fall applications gave best results. The sulfur fertilizers used were very stimulative of the root system, increasing its size and the number of nodules. The fertilized plants contained more sulfur, more protein, and more nitrogen than the unfertilized. Gypsum was equal to superphosphate in results, but it was expected that eventually the latter would give superior returns, because the phosphorus content of the soils experimented with was rather low. Rock phosphate gave negative results in this region.

1920. Stewart (54), from very slight increases in the yield of soybeans and alfalfa grown in the field, and from slight decreases in clover yields, over a period of years, concluded that sulfur is not a factor in the production of crops, on brown silt loam at least. After examining the results obtained with gypsum during a period of 18 years at the Ohio station, he concluded that it is quite evident that the apparently beneficial action of gypsum is due to its stimulating effect, particularly on bacterial life (shown by Greaves), thus enabling the crop to draw better upon the inadequate supply of phosphorus in the soil.

1920. Singh (53) found, by the use of pot cultures, that gypsum generally increased the process of fixation of nitrogen by *B. radicola*, the greatest increase occurring with the largest application. He further found that 1000 pounds of gypsum increased the yield of red clover, but that other applications did not have any effect on other legumes (alfalfa, Canada field peas, and soybeans). The nitrogen content of legumes, he found, was not affected by gypsum.

The literature upon the subject of the effect of fertilizer salts upon soil nitrification appears to be somewhat limited. A few citations having a bearing upon this phase of our investigation however may be stated.

1904. Fraps (11) pointed out that phosphoric acid and potash increased nitrification in some soils, while in other soils the opposite effect was produced.

1908. The same investigator (12) showed that these soil constituents had little effect upon the production of active nitrogen, though in some cases nitrification was affected considerably. With both phosphoric acid and potash the active nitrogen was much less affected than the production of nitrates.

1920. Fraps (13) also found that the addition of phosphate and of potash to potted soils increased nitrification in several types of soil and caused the soils which nitrify very slowly to nitrify in a shorter time. Dicalcium phosphate was more effective than potash (K_2SO_4) in these respects. He further showed that calcium carbonate increased nitrification. During these experiments, however, a considerable time elapsed before he noticed the formation of nitrates.

1909. Lipman (28) observed that the amounts of NO_3 nitrogen in parts per million were favorably affected by gypsum.

1912. Patterson and Scott (44) found that superphosphate increased nitrification of ammonia added to a soil, and concluded that this fertilizer may prove a useful aid to nitrification. The soil, however, was poor in P_2O_5 (0.032 per cent). They suggested that phosphates may help to nourish nitrifying organisms as well as the crop; and that where not required by these organisms, superphosphate, being acid, will probably do harm. Gypsum, they found, had a moderate effect in encouraging nitrification, but was not at all equal to calcium carbonate in this respect. They further showed that sodium chloride (salt) had a bad all-round effect on nitrate production.

1916. Jensen (24) found that bone meal, superphosphate, waste lime, and dry yard manure decreased the nitrifying activity in field soils. The manured plots lost most nitrogen, especially those to which ammonium sulfate was added, while the limed plots showed a gain in total nitrogen. Plots receiving calcium cyanamid, phosphatic fertilizers, and nitrate showed a slight gain in total nitrogen over the checks.

1916. Duley (8) showed that the nitrate content of the soil varied inversely with the amount of soluble sulfate in the soil.

1918. Fulmer (18) found that while nitrification is benefited by limestone, calcium carbonate and magnesium carbonate (particularly by the latter), it is only very slightly increased by phosphates (dibasic magnesium phosphate and monocalcium phosphate were used) in certain Wisconsin soils.

1918. Greaves (19) and his co-workers showed that calcium sulfate is more efficient than potassium chloride as a stimulator of nitrification, increasing nitric-nitrogen accumulation of the soil 97 per cent. They found that those compounds which are the strongest plant stimulants also are the most active in increasing nitric-nitrogen accumulation of the soil, and that it is very likely that the effect upon the plant is due mainly to the action of the compound upon the bacteria, which in turn render available more plant-food. They asserted, however, that the ammonifying powers of a soil containing alkalis are a better index to its crop-producing powers than are the nitrifying powers. They further found that nitrification was least with KCl out of the six chlorides experimented with. The soil, however, contained over 7 per cent of CaCO_3 , and therefore was suited for satisfactory nitrification results from the use of gypsum.

1920. Whiting and Schoonover (58), working with field soils in which soybeans were grown, showed that phosphorus in the form of rock phosphate increased nitric nitrogen to the extent of 18.09 to 19.01 pounds per acre, over and above that produced by organic matter (stable manure or crop residues).

1920. Singh (53), working with pot cultures, found that nitrification was depressed by gypsum alone, but the use of gypsum and lime together increased the process.

EXPERIMENTAL

Methods and results

Thirty-six square, stout wooden boxes were each filled with 128 pounds of a mixture composed of 110 pounds of clean sand and 18 pounds of a sandy loam soil. The soil medium was thus decidedly low in plant nutrients but contained enough to supply the crops grown provided it was in an available condition. This was designed to make very pronounced the effect of those fertilizer nutrients in the soil that were not readily available as compared with those that were. The inclusion of the loam served the purpose of introducing the nitrifying organisms. The subsequent crop growth was carried out in the greenhouse. The content of each box was compacted alike, and the moisture content of the soil, as far as possible, was maintained throughout at 10 per cent (on the dry-soil basis) by weighing the boxes at regular intervals, varying with the crop and with the stage of the growing season. On November 7 and 9, respectively, alfalfa and Canada field peas were each sown in 18 boxes containing 9 separate treatments, in duplicate. To each box was added $\frac{3}{4}$ pound of calcium carbonate, it having been shown by various investigators to promote assimilation of nitrogen by legumes. The varying treatments were as follows:

BOX NUMBER	TREATMENT
1, 10, 19, 28	No fertilizer (checks)
2, 11, 20, 29	Nitrogen (dried blood, 12 gm. per box)
3, 12, 21, 30	Phosphorus (disodium phosphate, 8 gm. per box)
4, 13, 22, 31	Potassium (muriate of potash, 8 gm. per box)
5, 14, 23, 32	Sulfur (gypsum, 8 gm. per box)
6, 15, 24, 33	Nitrogen, phosphorus, potash and sulfur in above forms (total 36 gm.)
7, 16, 25, 34	Nitrogen, phosphorus, and potash in above forms (total 28 gm.)
8, 17, 26, 35	Nitrogen, potash, and sulfur, in above forms (total 28 gm.)
9, 18, 27, 36	Phosphorus, potash, and sulfur, in above forms (total 24 gm.)

Previous to seeding, the boxes were inoculated with sand cultures containing the sub-species of *B. radicola* corresponding to the legume sown. In boxes 1 to 18 alfalfa was sown at the same rate as ordinarily sown under field conditions. The plants were subsequently thinned out to 23 per box. Boxes 19 to 36 were seeded with Canada field peas at the rate of 25 per box. These were later thinned out to 11 per box.

Because of backwardness in becoming established, due doubtless to an insufficient supply of nitrogen, the alfalfa seedlings were sprinkled on January 23, 1920, with a solution of nitrate of soda at the rate of 1.94 gm. per box (approximately 100 pounds per acre of 3,000,000 pounds of soil).

Five cuttings of alfalfa (cut when almost fully flowered, except in the case of cutting no. 5 which failed to flower because of the lateness and coolness of the season) were obtained. These were dried in the drying chamber, weighed and analyzed for dry matter and total nitrogen.

The peas, which produced an enormous growth, were carefully kept upright, and were harvested when fully ripe. The grain and straw were weighed, and analyzed for dry-matter and nitrogen, separately. Photographs of the pea growth are shown in plate 1.

Following the crop of Canada field peas, Ito San soybeans were seeded on May 22, 1920, after suitable inoculation of the soil. These were kept upright also and allowed to ripen fully before harvesting. The grain and straw were weighed, and separately analyzed for dry matter and nitrogen. .

The lime, in the form of calcium carbonate, was applied at the rate of 3 tons (of 2000 pounds) per acre of 3,000,000 pounds of soil, the salts at the rate of 282.6 pounds per acre, and the dried blood at the rate of 424 pounds per acre.

TABLE 1
Average total dry matter in the various crops for the various treatments

TREATMENT	ALFALFA (23 PLANTS, TOTAL OF 5 CUT- TINGS)	CANADA FIELD PEAS (11 PLANTS)			SOYBEANS (12 PLANTS)		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Lime alone (check).....	157.200	42.117	62.63	104.750	30.520	72.825	103.345
Lime and nitrogen.....	154.935	53.480	74.49	127.970	30.970	74.525	105.495
Lime and phosphorus.....	192.805	72.400	123.67	196.075	33.260*	88.920	122.180
Lime and potassium.....	163.490	50.217	72.36	122.580	29.735	69.635	99.370
Lime and sulfur.....	168.665	25.140	49.85	75.000	30.635	71.600	102.235
Lime, nitrogen, phosphorus, potassium and sulfur.....	209.925	77.665	125.40	203.070	35.435	86.165	121.600
Lime, nitrogen, phosphorus and potassium.....	197.690	77.250	130.62	207.870	32.205	85.940	118.145
Lime, nitrogen, potassium and sulfur.....	174.995	32.677	56.54	89.220	28.520	73.135	101.655
Lime, phosphorus, potassium, and sulfur.....	186.290	75.370	128.33	203.705	34.230	82.465	116.695

* Only one box included in average.

Following the harvesting of the soybeans and of the fifth cutting of alfalfa, the boxes of soil (plus roots) were incubated for three weeks at greenhouse temperatures, the moisture content at 10 per cent being maintained throughout this period. Immediately following incubation, the contents of the boxes were carefully sampled by making six full-depth borings with a soil auger in each box. These samples were immediately extracted with distilled water and the extracts analyzed for nitrate nitrogen by the colorimetric method.

In the determination of total nitrogen in the various crops the Kjeldahl-Gunning method was used throughout. These determinations were conducted for the most part in duplicate, but where wide or reasonably wide variations between the duplicates occurred (as happened in a few instances, especially in analyzing the grain) triplicate determinations were made and the

nearest two titrations were selected for averaging. The dry-matter and nitrate-nitrogen determinations also were conducted in duplicate.

Tables 1, 2 and 3 show the average weights of dry matter and of the total nitrogen, also the average nitrogen percentages (based on the dry matter) for the duplicate boxes growing the three crops under all treatments.

Table 4 gives the averaged nitrification results from all salts, including lime (checks), after the growth of crops.

Tables 5, 6 and 7 record the percentage increases of dry matter, of total nitrogen, and of the percentage of nitrogen in the three legumes as the result of soil treatment with the above-mentioned nutrient salts. From these tables and tables 8 and 9 the conclusions enumerated at the close of this thesis have been drawn.

TABLE 2
Average total nitrogen in the various crops for the various treatments

TREATMENT	ALFALFA (5 CUT- TINGS)	CANADA FIELD PEAS			SOYBEANS		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Lime alone (check).....	5.300	1.895	0.605	2.500	2.170	0.910	3.080
Lime and nitrogen.....	5.315	2.480	1.010	3.490	2.340	0.890	3.230
Lime and phosphorus.....	6.925	3.450	1.705	5.155	2.690	1.550	4.240
Lime and potassium.....	5.700	2.750	1.110	3.860	2.210	0.810	3.020
Lime and sulfur.....	5.645	1.235	0.860	2.095	2.240	0.815	3.055
Lime, nitrogen, phosphorus, potassium and sulfur.....	7.335	3.645	1.740	5.385	2.605	1.390	3.995
Lime, nitrogen, phosphorus and potassium.....	7.065	3.780	2.090	5.870	2.520	1.460	3.980
Lime, nitrogen, potassium and sulfur.....	5.930	1.560	1.055	2.615	2.105	0.995	3.100
Lime, phosphorus, potassium and sulfur.....	6.570	3.470	2.230	5.700	2.585	1.225	3.810

Tables 8 and 9 show the actual and percentage increases of nitrate nitrogen, in parts per million, after the growth of alfalfa and of Canada field peas and soybeans by the various nutrient salts. Dry-matter increases (actual) also are included for comparison with the corresponding nitrate-nitrogen increases.

During the growth of the legumes a few notes of special interest respecting the behavior of the plants were made from time to time.

In the peas the potash-treated plants were the first to flower, blossoms being noticed on the tall phosphorus-treated plants some two days later. Where potash was supplied the pods appeared to be best filled, while plants without a potash supply seemed insufficiently filled. Where a complete fertilizer was added the pods were more advanced and the vines ripened before those in the other boxes.

In the alfalfa the plants that received phosphorus flowered first and thereon the flowers were the most abundant. The accelerating effect of phosphorus on the reproductive parts of the crop was here demonstrated.

In the first growth of alfalfa an apparently injurious effect of sulfur was somewhat noticeable, but in later cuttings this was not visible. The inhibiting action on growth, more especially where sulfur was used alone, had disappeared, as is recorded in the percentage increases for the second and subsequent cuttings. On the other hand, this effect of sulfur used alone on the peas was visible throughout the growth of the crop.

TABLE 3
Average percentage of nitrogen in the various crops for the various treatments

TREATMENT	ALFALFA (5 CUT- TINGS)	CANADA FIELD PEAS			SOYBEANS		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Lime alone (check).....	3.420	4.480	0.960	2.720	7.125	1.240	4.18
Lime and nitrogen.....	3.490	4.640	1.360	3.000	7.525	1.200	4.36
Lime and phosphorus.....	3.616	4.765	1.385	3.075	8.090	1.750	4.92
Lime and potassium.....	3.559	4.530	1.535	3.032	7.430	1.160	4.29
Lime and sulfur.....	3.415	4.825	1.795	3.310	7.305	1.145	4.22
Lime, nitrogen, phosphorus, potassium and sulfur.....	3.555	4.695	1.360	3.027	7.335	1.610	4.47
Lime, nitrogen, phosphorus and potassium.....	3.611	4.890	1.610	3.250	7.845	1.710	4.78
Lime, nitrogen, potassium and sulfur.....	3.479	4.825	1.870	3.347	7.375	1.355	4.36
Lime, phosphorus, potassium and sulfur.....	3.600	4.600	1.735	3.167	7.555	1.485	4.52

TABLE 4
Average nitrate nitrogen in the soil after removal of crops, for the various treatments

TREATMENT	NO ₃ IN DRY SOIL		REMARKS
	After alfalfa	After Canada field peas and soybeans	
	<i>p. p. m.</i>	<i>p. p. m.</i>	
Lime alone (checks).....	4.95	10.7	Nitrification determinations after Canada field peas and soybeans, grown successively, had the advantage of the well rotted root system of the pea crop
Lime and nitrogen.....	6.85	11.4	
Lime and phosphorus.....	10.85	13.2	
Lime and potassium.....	4.50	7.4	
Lime and sulfur.....	5.85	8.2	
Lime, nitrogen, phosphorus, potassium, and sulfur.....	8.80	15.7	
Lime, nitrogen, phosphorus and potassium	13.65	22.5	
Lime, nitrogen, potassium, and sulfur....	11.55	10.5	
Lime, phosphorus, potassium and sulfur..	8.55	18.0	

Discussion of crop results

Upon referring to tables 1 to 3 and 5 to 7, it will be at once noticed that phosphorus has produced the most marked effect of all of the elements applied. The effect of phosphorus in increasing the dry matter, total nitrogen, and also, although to a lesser extent, the percentage of nitrogen in the legumes grown, is unmistakable. The literature cited above substantiates these find-

TABLE 5

Percentage increases of total dry matter over the checks, due to various treatments, for the three legumes*

TREATMENT	ALFALFA (TOTAL OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen.....	-1.504	22.167	2.080
Phosphorus.....	22.571	87.183	18.225
Potassium.....	3.935	17.021	-3.847
Sulfur.....	7.225	-28.401	-1.074
Nitrogen, phosphorus, potassium and sulfur....	33.455	93.861	17.664
Nitrogen, phosphorus and potassium.....	25.677	98.443	14.321
Nitrogen, potassium and sulfur.....	11.249	-14.826	-1.636
Phosphorus, potassium and sulfur.....	18.429	94.467	12.917

* Checks received lime alone and all treatments contained lime at the same rate.

TABLE 6

Percentage increases of total nitrogen over the checks, due to various treatments, for the three legumes

TREATMENT	ALFALFA (TOTAL OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen.....	0.283	39.6	4.870
Phosphorus.....	30.660	106.2	37.662
Potassium.....	7.547	54.4	-1.948
Sulfur.....	6.509	-16.2	-0.812
Nitrogen, phosphorus, potassium and sulfur....	38.396	115.4	29.707
Nitrogen, phosphorus and potassium.....	33.301	134.8	29.202
Nitrogen, potassium and sulfur.....	11.886	4.6	0.649
Phosphorus, potassium and sulfur.....	23.962	128.0	23.701

ings with respect to the beneficial influence of phosphorus; and these results add further testimony to the importance of this vital substance to the growth of crops and to the growth of leguminous crops in particular. Doubtless, this decidedly beneficial influence is due mainly to the bacterial stimulus by phosphorus, as is indicated by Truesdell (56).

With legumes, this experiment has indicated that any fertilizer, possibly with the exception of sulfur, that increases yield increases the percentage of nitrogen.

Naturally, combined nitrogen is not as essential to legumes as is phosphorus. Nevertheless, we find it playing some part in the growth of these crops, varying with the crop and its habit of growth and with the association of elements in which nitrogen is employed. For example, of the three plant species, peas were benefited in growth the most by nitrogen when it was used alone, while alfalfa was the least benefited; whereas by nitrogen in combination with other substances alfalfa was benefited in growth the most, and peas the least. While nitrogen, used alone, here slightly increased the percentage of nitrogen in the three legumes, particularly in the case of peas, yet when used in combination with other substances it did not have this effect.

In general, combined nitrogen in this experiment appeared to play some part in promoting nitrogen assimilation by legumes. It at least did not hamper the operation of this phenomenon, in keeping with the findings of the

TABLE 7

Percentage increases of percentage of nitrogen in plants over the checks, due to various treatments, for the three legumes

TREATMENT	ALFALFA (AVERAGE OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen.....	2.046	10.294	4.306
Phosphorus.....	5.731	13.051	17.703
Potassium.....	4.064	11.489	2.631
Sulfur.....	-0.146	21.691	0.957
Nitrogen, phosphorus, potassium and sulfur....	3.947	11.305	6.937
Nitrogen, phosphorus and potassium.....	5.584	19.485	14.354
Nitrogen, potassium and sulfur.....	1.725	23.069	4.306
Phosphorus, potassium and sulfur.....	5.263	16.452	8.134

majority of the investigators cited under this section; but whether or not the action would be impaired in the presence of large quantities of nitrogen is not within the scope of this investigation to answer.

The treatments were so arranged that only the effects of potassium used alone can be considered and these effects are beneficial in the cases of the growth of peas and of alfalfa, but apparently not in the case of the growth of soybeans. Peas were the most benefited in growth by muriate of potash, for this crop, of all three crops, showed the largest percentage increases of dry matter, of total nitrogen, and of percentage of nitrogen with potassium treatment.

Sulfur, without other fertilizer substances and in the form of gypsum, was apparently toxic to peas and slightly toxic to soybeans. To alfalfa, however, it proved beneficial, and this effect increased with the development of the crop, as shown by the successive cuttings, doubtless because of the disappearance of the toxic influence at first established in the soil. Had a less sandy soil been used the seemingly toxic effect noted, in all probability, would have

been less in evidence. Sulfur in combination with other substances was apparently toxic only in the case of peas, and even here this seeming toxicity was less marked than it was where sulfur was used alone. The fact that the treatments contained lime in fair quantity may possibly have accounted, in no small measure, for the satisfactory results obtained with alfalfa when fertilized with calcium sulfate—an experience recorded from experiments embodying the use of gypsum on calcareous soils.

As shown by Truesdell (56) in his investigations, the third cutting of alfalfa, on the whole, was the most satisfactory, the yields of dry-matter and the analyses being in general higher than those associated with the other cuttings.

The striking differences for the various treatments shown throughout the investigation have been made possible as the result mainly of using a compounded soil that was practically a sand. Had an ordinary soil been used, these differences would in large measure have been masked by the effect of plant-food elements inherent in the soil. The results herein obtained can at least lay claim to have in some small measure strengthened our knowledge of the growth requirements of legumes, and of alfalfa, Canada field peas and soybeans in particular.

TABLE 8
Increase in soil nitrification due to salts, after growth of alfalfa (5 cuttings)

INCREASE DUE TO	INCREASE OF NO ₃ OVER CHECK		INCREASE OF TOTAL DRY MATTER
	p. p. m.	per cent	gm.
Nitrogen.....	1.90	38.38	-2.365
Phosphorus.....	5.90	98.98	35.505
Potassium.....	-0.45	-9.09	6.190
Sulfur.....	0.90	18.18	11.365
Nitrogen, phosphorus, potassium and sulfur....	3.85	77.77	52.625
Nitrogen, phosphorus and potassium.....	8.70	175.75	40.390
Nitrogen, potassium and sulfur.....	6.60	133.33	17.695
Phosphorus, potassium and sulfur.....	3.60	72.72	28.990

TABLE 9
Increase in soil nitrification due to salts, after growth of Canada field peas and soybeans

INCREASE DUE TO	INCREASE OF NO ₃ OVER CHECK		INCREASE OF TOTAL DRY MATTER	
			Peas and soybeans	Peas alone
	p. p. m.	per cent	gm.	gm.
Nitrogen.....	0.7	6.54	25.370	23.220
Phosphorus.....	2.5	23.36	110.160	91.325
Potassium.....	-3.0	-28.03	13.855	17.830
Sulfur.....	-2.5	-23.36	-30.860	-29.750
Nitrogen, phosphorus, potassium and sulfur....	5.0	46.72	116.575	75.100
Nitrogen, phosphorus and potassium.....	11.8	110.28	117.920	103.120
Nitrogen, potassium and sulfur.....	-0.2	-1.87	-17.220	-15.530
Phosphorus, potassium and sulfur.....	7.3	68.22	112.305	98.955

Discussion of soil nitrification results

A perusal of the soil nitrification results, as recorded in tables 4, 8, and 9, shows that salts or their combinations which most markedly promoted the growth of legumes usually caused the highest nitrification. Such was particularly the case wherever phosphorus was applied. This observation concurs with the conclusion of Greaves (19) who found that those compounds which are the strongest plant stimulants are also the most active in increasing nitric-nitrogen accumulation in the soil. He attributes this correlation to the stimulus given to the bacteria by the beneficial compound. This may be a factor in the results herein recorded, but we are inclined to give some recognition also to the effect of the decayed roots of the previous crop upon nitric-nitrogen accumulation. The increased top growth is correlated with increased root development, hence with more organic matter for nitrification. There was greater nitrification after peas and soybeans (grown in the same boxes of soil) than after alfalfa (five cuttings). The extensive root systems of the huge pea plants had opportunity to decay well, whereas there would be less decay of the alfalfa roots, even though extensive.

Nitrogen, applied alone, increased soil nitrification after all three crops, particularly after alfalfa; but when this nutrient was applied in combination with the other substances, it decreased nitrification after peas and soybeans and slightly increased it after alfalfa. It would thus appear that alfalfa is less dependent upon nitrate nitrogen for growth than are the other two legumes, peas especially.

Sulfur depressed nitrate-nitrogen accumulation, except when used alone as a fertilizer nutrient for alfalfa, which crop it also otherwise benefited, both alone and combined with other elements. In general, this finding was in accordance with the findings of Duley (8) who found that the nitrate-content of the soil varied inversely with the amount of soluble sulfate in the soil.

Potassium apparently slightly inhibited nitrate-accumulation after all three crops. It may here be mentioned, however, that because of the presence of chlorides (in the KCl used) there may possibly have been a slight loss of nitrates during the process of determination by the colorimetric method, which involves the use of pheno-disulfonic acid.

CONCLUSIONS

Effects of phosphorus

Of all the fertilizer elements in the salts applied to the compounded soil, phosphorus showed the most marked effect. As a single element it markedly increased the dry-matter and total nitrogen, and to a lesser extent the percentage of nitrogen in all three legumes, the order of greatest average influence on the crop being: (a) Canada field peas, (b) soybeans and (c) alfalfa. In the three crops phosphorus, used alone, showed its powerful influence on the three

factors in the following order: (a) increase of total nitrogen; (b) increase of dry-matter and (c) increase in the percentage of nitrogen.

In combination with nitrogen, potassium and sulfur, phosphorus markedly increased the dry matter and total nitrogen in Canada field peas, soybeans and alfalfa. However, it increased the percentage of nitrogen in soybeans and alfalfa only slightly, if at all, and decreased the percentage in the case of peas.

Effects of nitrogen

As a single element nitrogen can hardly be said to benefit the plants with respect to yields of either dry matter or nitrogen, or the percentage of nitrogen, unless in the case of Canada field peas, which appeared to respond somewhat in each of these three properties.

In combination with phosphorus, potassium, and sulfur, nitrogen promoted no more response in the legumes than where it was employed alone. Indeed, there was perhaps less response from nitrogen when used in association with the other elements.

Combined nitrogen did not hamper the operation of nitrogen assimilation by legumes; but whether or not it would have hindered the phenomenon had large quantities of nitrogen been used, could not be answered by this experiment.

Effects of potassium

Potassium, used alone, showed its greatest influence in increasing, on the average, the total nitrogen and dry matter in Canada field peas and alfalfa, in the order named. In soybeans, however, it showed a decrease with respect to these factors. Only in the percentage of nitrogen did potassium show an increase common to all three crops, and this in the crop order just named.

Effects of sulfur

Sulfur, in the form of gypsum, used alone and in combination with other fertilizer salts, increased somewhat the growth and nitrogen content of alfalfa but appears not to have had any effect on field peas and soybeans.

General effect of fertilizer salts

In general it may be said that when any application of fertilizer, with the exception of gypsum, increased the yield of the legumes grown, there was also an increase in the percentage of nitrogen in the plants.

Effects of fertilizer salts on soil nitrification, after legumes

Where phosphorus was applied there was, in general, the greatest nitrate accumulation after all crops. Thus salts or their combinations which most markedly promoted the growth of legumes, as did phosphorus, usually caused the greatest nitrification.

Nitrogen applied alone increased soil nitrification after all three crops, particularly after alfalfa, but when this nutrient was applied in combination with the other substances it did not have such an effect.

Potassium, in the form of muriate of potash, apparently slightly inhibited nitrate-nitrogen accumulation.

Sulfur, in the form of gypsum, increased nitrification in soil in which alfalfa had grown but not in soil in which peas and soybeans had grown. There appears to be a connection between the effect of sulfur on the crop and on nitrification following the crop.

In general, there appeared to be a tendency toward correlation between the dry matter produced and subsequent soil nitrification—due in part, it is assumed, to the greater root system associated with greater top growth, hence to greater amounts of decayed roots for promoting nitrification.

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PLATE 1

FIG. 1. Canada field peas fertilized with individual elements; note the pronounced effect of phosphorus.

FIG. 2. Canada field peas fertilized with elements in various combinations; note the pronounced effect of phosphorus.

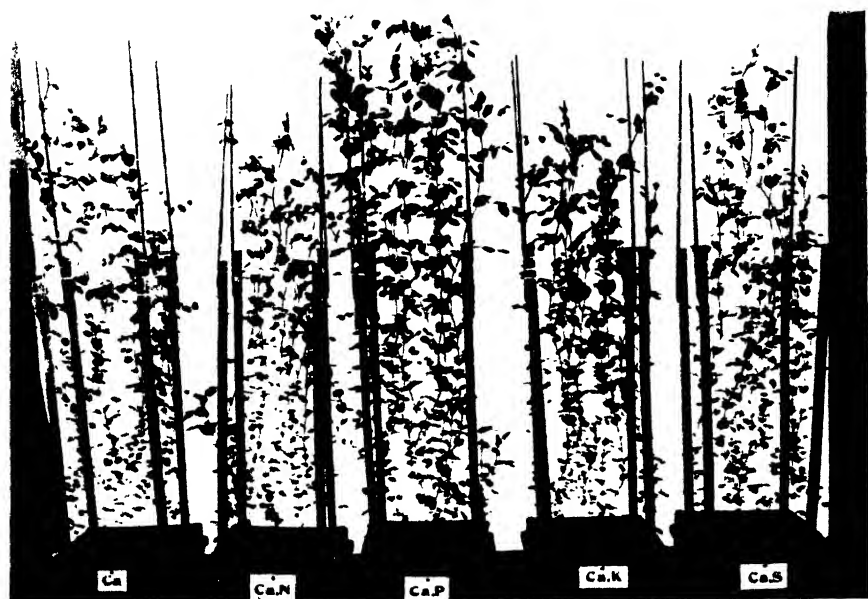


FIG. 1

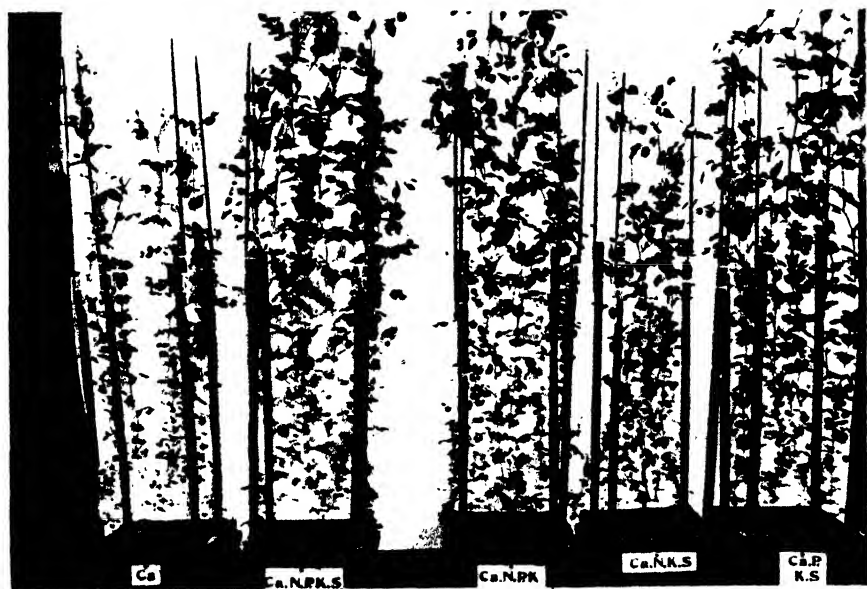


FIG. 2

STUDIES ON THE ACID AMIDE FRACTION OF THE NITROGEN OF PEAT. I¹

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During the past decade a large amount of work has been done on the distribution of the organic nitrogen in soil organic matter. In this laboratory special attention has been paid to the composition of peat soils. The result of the work has been to show that "in comparison with ordinary proteins, peat contains a larger percentage of acid amide and humin nitrogen and a smaller amount of basic and non-basic nitrogen compounds" (7).

In a general way the total nitrogen in peat is divided as follows:

	<i>per cent of total N</i>
Humin nitrogen	2.0-8.0
Ammoniacal nitrogen	0.5-0.8
Acid amide nitrogen	10.0-20.0
Non-basic amino nitrogen	22.0-40.0
Non-basic non-amino nitrogen	3.0-10.0
Basic amino nitrogen	3.5- 6.0
Basic non-amino nitrogen	1.5- 6.5

It will be observed from these figures that the largest two fractions are the acid amide and non-basic amino or mono amino acid fractions. In pursuance of a plan outlined some time ago the former is now receiving special attention in our work.

Before proceeding further with the discussion it might be well to state just what is meant by the acid amide fraction as applied to peat. By this term is meant the nitrogen that is evolved as ammonia upon treatment with acid and subsequent distillation after making alkaline. When dealing with pure protein material the statement is justified that this truly represents the nitrogen present in the form of acid amides. It has been contended, and the authors admit the contention, that in a complex and little known material like peat it is not safe to apply the same criteria to the various subdivisions obtained that are used in the treatment of pure proteins. Hence it must be understood that the term "acid amide fraction" as used in this work refers to a fraction showing the same behavior that acid amides do in proteins, but that

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in peat it may be composed of other sorts of compounds. In other words, strictly speaking it is a term of convenience. It is true that there is no definite idea as to what these compounds might be and from the work of Jodidi (3) the preponderance of such evidence as we have is against their existence, but, nevertheless, in view of the uncertainty of our knowledge of the constitution of peat, it seems advisable to accept the possibility.

THE SIGNIFICANCE OF ACID AMIDES IN SOILS

The value of organic nitrogen compounds as sources of nitrogen for plant nutrition depends largely upon the ease with which they are converted into ammonia. From the readiness with which acid amides can be made to split off ammonia in vitro it would seem to be a logical conclusion that in the soil this fraction would be the one to become ammonified most rapidly, i.e. would be the most "available." An attempt was made to connect this fraction with the availability of nitrogen in peat as determined by the alkaline permanganate method but with small success, and the presence of such a large percentage of nitrogen in this form also affords an indication that it is not as easily decomposed as would be anticipated on the above grounds.

Actual experimental data on the behavior of acid amides in soils have been obtained by several investigators, notably Jodidi, Kelley and Lathrop.

Jodidi (3) added weighed amounts of amino acids and acid amides to definite quantities of soil and determined the ammonia produced. He found that the results depended upon the structure of the particular compounds used but that acid amides and amino acids did not differ materially as classes so far as the rapidity of their ammonification in soil was concerned.

Kelley (4) found that the basic nitrogen fraction of the materials investigated was more easily ammonified than either the non-basic or acid amide fractions. As regards the last two groups sometimes one and sometimes the other was decomposed more rapidly.

The most exhaustive work on the subject is that of Lathrop (5, 6), who studied the changes in the various fractions of nitrogen in dried blood when added to soil. He found that during the early stages the amide nitrogen diminished very rapidly. It then increased to a maximum after which it slowly decreased in quantity. The conclusion that Lathrop drew from his work was that through the action of microorganisms the dried blood was destroyed with the synthesis of a new protein which was much more resistant to decomposition than was the original dried blood.

Summarizing the results that have been obtained up to the present it may be said that soil organic matter contains material of a protein nature formed from the decomposition of protein which differs from the latter in having a high content of amide nitrogen compounds. These compounds differ from the acid amides ordinarily found in proteins in that they are very resistant to ammonification by the agents which are usually efficient in causing this. So

pronounced is this characteristic that these compounds may actually be produced in soils which exhibit a very active ammonifying power on the amides of ordinary proteins added to them.

The question which at once presents itself is, Why are the amides of soil organic matter so different from those of proteins in this respect? It has been shown that there is apparently a close relationship between the amide nitrogen contents and the amounts of the dibasic acids glutaminic and aspartic, which are present in proteins. The recent work of Dakin (1) who, in using Foreman's (2) method of separation, found that caseinogen contained a much larger amount of glutaminic acid than had formerly been obtained, will necessitate a revision of the theories on this subject, at least so far as this particular protein is concerned. His discovery of a third dibasic acid, hydroxyglutaminic acid, also tends to complicate the matter. Whether or not these same factors enter into the situation as respects other proteins is as yet unknown and in order to test it out in the case of soil organic matter which contains such an unusually large amount of amide nitrogen we are attempting to determine the relative amounts of the dibasic acids in peat.

That material of the nature of that under investigation contains some glutaminic and aspartic acids is known from the work of Suzuki (8) who isolated both from the hydrolytic products of humus by the esterification method. So far as we know, however, there has never been any attempt made to estimate them quantitatively.

In the course of the extensive work which has been carried on in this laboratory with peat soils, repeated attempts have been made to isolate glutaminic acid as the hydrochloride directly from the hydrolysates. The ease with which it can be obtained from the hydrolysates of many proteins naturally tempted us to try to get it from our material. The following experiments are typical of the methods of procedure employed and the results obtained.

Three hundred grams of oven-dried brown peat were digested with 1500 cc. of concentrated HCl for 16 hours on the hot plate. The product was filtered and the residue boiled with water and filtered through a Büchner funnel, the process being repeated several times. The combined filtrates were then concentrated to a volume of about 100 cc. by the method used in previous work. During this process crystals of calcium sulfate separated out and were removed by filtration. They were probably formed from sulfuric acid carried over from the drying flasks. The resultant solution was saturated with dry hydrochloric acid gas at 0° and allowed to stand some days in the refrigerator. A precipitate of inorganic matter had separated out at the end of this time, which was removed and the filtrate concentrated to a thin sirup. It was again saturated with hydrochloric acid and placed in the refrigerator after being inoculated with crystals of glutaminic acid hydrochloride. A second crop of inorganic crystals resulted. These were removed, the filtrate and washings concentrated, inoculated and placed in the ice box but even on long standing no crystals separated.

It was apparent from this and similar experiments that either there was too little glutaminic acid present to be separated in this way or the conditions for its crystallization were not proper, as a result, perhaps, of the presence of some inhibiting substance. In this connection the following quotation from Foreman (2) is of interest:

The ease with which glutaminic acid hydrochloride separates seems to vary with the protein under treatment and with the amount of glutaminic acid present. Plimmer (1912) states that separation of the hydrochloride "occurs in the case of caseinogen and certain vegetable proteins which contain from 10 to 40 per cent of this amino acid." If the protein contains under 10 per cent, then as a rule, no hydrochloride will separate. Occasionally, however, a separation is obtained; thus Hopkins and Savery (1911) working with Bence-Jones protein obtained 8.05 per cent.

To shed some light on this point 100 gm. of oven-dried peat was mixed with 30 gm. of casein and boiled with 500 cc. of concentrated hydrochloric acid on the hot plate for 48 hours. The mass was filtered through asbestos and washed by repeatedly boiling the residue with water and sucking it dry on a Büchner funnel. The filtrate and washings were combined and concentrated at 50° until a sirupy consistency was reached. This sirup was saturated at 0° with dry hydrochloric acid gas and after inoculation with a few crystals of glutaminic acid hydrochloride was set in the ice box and allowed to stand several days. A precipitate formed which was filtered on asbestos and washed with cold hydrochloric acid. After one recrystallization from hydrochloric acid it was pure white and melted at 197° (uncor.). It weighed 3.97 gm., representing a yield of 13.2 per cent.

It was thus made apparent that with the relative quantities of peat and casein used fairly good returns of glutaminic acid could be expected on the basis of that contained in the casein alone. The indications were that the amount present in the peat must be small. An attempt was next made to determine approximately the limits of precipitation for glutaminic acid from hydrolysates of peat.

Ten grams of casein and 187 gm. of peat were hydrolyzed and worked up in the usual manner, the filtrate and washings being concentrated to a volume of 20 cc. As a control 10 gm. of casein were treated in the same manner. The hydrolysates were saturated with dry hydrochloric acid gas and allowed to stand in the refrigerator for 4 days.

The precipitate from the casein alone weighed 1.45 gm. and melted at 197.5°. A second crop of 0.033 gm. melting at 195° also was obtained. This yield compared well with the results of Osborne and Guest which were the highest obtained up to the time of this work. The conditions seemed to be favorable for the maximum precipitation of the acid.

The precipitate from the peat-casein mixture, however, weighed 8.12 gm. and was largely inorganic, containing the chlorides of iron, calcium and magnesium. It was dissolved in distilled water and boiled with a slight excess of sodium carbonate to precipitate the heavy metals. The precipitate was

filtered off and the filtrate acidified with hydrochloric acid. The greater part of the sodium chloride was precipitated by passing in dry hydrochloric acid gas without cooling the solution. The first crop of sodium chloride thus obtained carried with it no organic matter, but succeeding crops carried an increasing proportion of organic matter. When the glutaminic acid hydrochloride was obtained, however, it still held an appreciable quantity of salt.

The possibility of precipitating the inorganic chlorides first by omitting the inoculation with glutaminic acid hydrochloride was next attempted. Ten grams of casein and 200 gm. of peat were mixed, hydrolyzed and worked up in the usual manner. After concentration the extract was placed in the refrigerator without inoculation. Two crops of inorganic crystals were obtained. The mother liquor was saturated with hydrochloric acid gas, seeded with glutaminic acid hydrochloride and set in the ice box for a week. The precipitate which separated out was filtered, dissolved in distilled water and boiled with an excess of barium hydroxide to drive off ammonia. The excess barium was then removed quantitatively with sulfuric acid, the solution made up to a volume of 100 cc. and the amino nitrogen determined on an aliquot, with the following results: 1 cc. gave 1.72 cc. nitrogen at 23° and 742 mm.

This corresponds to 1.24 gm. of glutaminic acid hydrochloride, a yield slightly lower than that obtained from casein alone.

It was evident that with the quantities present in the last series of experiments considerable difficulty would be experienced in getting satisfactory yields of glutaminic acid by the direct method. Further attempts along this line of attack were discontinued without applying the results of the above work to peat alone because our attention was called to Foreman's method of isolating both glutaminic and aspartic acids which offered greater possibilities than did our own method.

Foreman's method depends upon the insolubility of the calcium salts of the dibasic acids in alcohol and has afforded very valuable results in the hands of its originator. Dakin has also used it to good advantage. As usual, in applying to peat a method which has been developed for use with pure proteins, difficulties were encountered which made deviations from the original procedure necessary. However, we were able to accomplish our purpose and have isolated both glutaminic and aspartic acids from peat. The technic has not yet been perfected to the point where conclusions regarding the quantitative relationships can be drawn, but the results are promising for the early accomplishment of this end.

Foreman found that upon adding an excess of lime to the hydrolysate of caseinogen, filtering it and adding alcohol to the filtrate, a precipitate was formed which consisted of the following fractions:

- (a) Glutaminic and aspartic acids.
- (b) A gummy substance very difficult to crystallize.

(c) A very small quantity of pigmented substance precipitated from the solution by silver sulfate solution.

(d) A substance precipitated by phosphotungstic acid.

The general method of procedure consisted in general in separating the hydrolysate into these fractions.

The first trial was made with a mixture of 10 gm. of casein and 200 gm. of peat, the same quantities used in the second set of preliminary experiments described above. The mixture was hydrolyzed by boiling with a liter of concentrated hydrochloric acid and worked up as in the preceding cases. The sirup resulting from the concentration of the filtrate and washings from the insoluble residue was taken up in about 400 cc. of water and treated with a cooled paste of calcium hydroxide. After shaking thoroughly the precipitate was filtered off and washed free from chlorides by repeated suspension in water and filtering. The combined filtrate and washings were concentrated *in vacuo* until a fairly concentrated but not sirupy solution was obtained. This was transferred to a 2-liter flask and alcohol (about 97 per cent) added in small portions at a time, the mixture being shaken thoroughly after each addition. A black sticky precipitate formed on the bottom and sides of the flask. It was filtered through a Büchner funnel and washed as thoroughly as possible with alcohol. After dissolving it in about 300 cc. of water the calcium was removed by means of oxalic acid. The chlorides and some of the coloring matter were then removed by means of silver sulfate solution. After filtering off the precipitate the excess silver was removed by passing hydrogen sulfide through the solution. The filtrate from the silver sulfide was concentrated *in vacuo* until all of the hydrogen sulfide had been removed. Phosphotungstic acid was then added until precipitation was complete. The solution at this point was still strongly colored. After filtering off the precipitate the excess phosphotungstic acid was removed by means of barium hydroxide and the excess barium in turn quantitatively removed by means of sulfuric acid. The filtrate from the barium sulfate was concentrated as far as possible by distillation *in vacuo* and then transferred to a weighed evaporating dish and dried over phosphorous pentoxide.

In the dry state the residue weighed 11.068 gm. and contained a large quantity of inorganic matter. It was dissolved in water, treated with an excess of barium hydroxide, filtered and the excess barium removed as before. This time the dried residue weighed 9.4907 gm. It was a hard gritty mass and very difficult to triturate with glacial acetic acid which was the next step in the process and required most of a day. The acetic-acid-insoluble residue when dried weighed 4.4055 gm. It was dissolved in water and made up to a volume of 200 cc.

The acetic-acid-soluble part was freed from acetic acid by distillation *in vacuo*. The gummy residue was dissolved in water and made up to a volume of 50 cc.

Then 100 cc. of the solution of the acetic-acid-insoluble part was boiled with an excess of copper carbonate. The filtrate from the excess copper

carbonate was concentrated *in vacuo* and from it on standing overnight was obtained a large crop of fine light blue needles. These were filtered from the mother liquor which was further concentrated but it yielded no more crystals. Two crops of crystals were obtained on recrystallizing the product from hot water. They weighed, respectively, 0.2917 and 0.1055 gm., making a total of 0.3972 gm. from the 100 cc. used, or 0.7944 gm. from the total sample of peat casein mixture. This corresponds to 0.5420 gm. of aspartic acid.

After removing the copper by means of hydrogen sulfide from the filtrate from the copper aspartate and distilling off the excess hydrogen sulfide *in vacuo*, the residue was dried in a desiccator over sulfuric acid. It weighed 1.9211 gm. This residue was boiled with concentrated hydrochloric acid, cooled and saturated with dry hydrochloric acid gas, after filtering off the brown precipitate that formed during the boiling with hydrochloric acid. After inoculating with a few crystals of glutaminic acid hydrochloride the solution was allowed to stand in the refrigerator for 3 days. The perfectly white crystals were filtered on asbestos. They weighed 0.6234 gm. and melted at 197°, being apparently quite pure. A second crop of less pure product was obtained, which after treatment with animal charcoal melted at 196° and weighed 0.0307 gm. The total amount of pure glutaminic acid hydrochloride in the hydrolysate of the peat-casein mixture calculated from these yields was 1.31 gm., corresponding to 1.05 gm. of glutaminic acid. This, of course, does not include that in the mother liquors from the original crystallization and from the recrystallizations. These solutions were transferred to a 100-cc. flask and made up to volume. An analysis for amino nitrogen gave the following results: 1 cc. gave 1.84 cc. nitrogen at 23.5° and 749 mm.

The total amino nitrogen content based on these figures was 0.1017 gm. for the 100 cc., or 0.2034 gm. for the whole hydrolysate. This corresponds to 2.66 gm. of pure glutaminic acid hydrochloride. Undoubtedly there was much glutaminic acid present, but as no attempt was made to remove the ammonia before making the above analysis the figures are not entirely accurate.

The next step was to try out the method on peat alone. As over half of the above quantities of glutaminic and aspartic acids could be accounted for by the presence of the 10 gm. of casein, it was decided to use a sufficient quantity of peat to insure getting measurable amounts of these compounds. Accordingly, 3 kgm. of peat was hydrolyzed as usual with a solution of 9 liters of concentrated hydrochloric acid and 6 liters of water, this giving approximately a constant boiling mixture. The resulting hydrolysate was concentrated *in vacuo* to about a liter, cooled and saturated with dry hydrochloric acid gas. Three crops of inorganic crystals were obtained, the last mother liquor being diluted to about 2 liters and treated with a cooled paste of calcium hydrate. About 125 gm. of CaO were required to give an excess. The large gelatinous precipitate produced was washed free from chlorides and the filtrate and washings concentrated *in vacuo* to about 500 cc. As the

resulting solution was rather viscous 100 cc. of water was added to it and then alcohol in small portions. A black, sticky precipitate was formed and was not completely thrown down by 5 liters of alcohol. However, at this point it was filtered off and the alcohol distilled from the filtrate, which was then further concentrated. Alcohol was then added to it, but precipitation was still incomplete when 5 liters had been added. The precipitate, however, was filtered off at that point, the alcohol distilled from the filtrate and the liquid again concentrated. It was taken up in a small quantity of water and added in small amounts to comparatively large volumes of alcohol, the resulting precipitates being filtered through the same filter.

The precipitates were combined, washed as thoroughly as possible, dissolved in water and freed from calcium by means of oxalic acid. Coloring matter and chlorides were removed with warm silver sulfate solution, the excess silver precipitated with hydrogen sulfide and the filtrate concentrated *in vacuo*.

Phosphotungstic acid was then added until precipitation was complete, a large quantity being required. The excess was removed as usual by means of barium hydrate and the excess of the latter was precipitated quantitatively by sulfuric acid. The final filtrate was concentrated by distillation *in vacuo* and finally dried in a weighed casserole in a vacuum desiccator over sulfuric acid and P_2O_5 . The resulting product was a hard brittle mass weighing 91.8 gm.

Considerable difficulty was experienced in extracting this material with acetic acid, but finally after triturating it in a porcelain casserole with small portions of acid, 41 gm. of a grey powder was obtained.

The extract was concentrated *in vacuo* to a stiff gum and placed in a vacuum desiccator over KOH. Even after standing thus for 5 months it still smelled strongly of acetic acid.

SEPARATION OF GLUTAMINIC AND ASPARTIC ACIDS

The acid-insoluble grey powder contained 41.59 per cent of ash, which consisted apparently of oxides of tungsten, and 8.5 per cent of amino nitrogen figured on the ash-free basis. Two grams of it was dissolved in water, treated with barium hydrate to remove ammonia and the excess barium removed quantitatively with sulfuric acid. After treating with bone-black it was concentrated *in vacuo* and made up to approximately a liter. This solution was treated with an excess of copper carbonate and filtered. The filtrate and washings were concentrated to 500 cc. *in vacuo* and allowed to stand overnight. Light blue needles separated, and were filtered, dried and weighed. The yield was 0.4609 gm.

The filtrate was freed from copper and concentrated *in vacuo* to about 15 cc. It was saturated in the cold with hydrochloric acid, inoculated with glutamic acid hydrochloride and allowed to stand several days in the ice box.

A crop of 0.1248 gm. of a white crystalline compound melting at 197° was obtained. The above work was now repeated with 25 gm. of the acetic-acid-insoluble substance. From this amount of material 5.716 gm. of the copper salt of aspartic acid and 2.00 gm. of glutaminic acid hydrochloride were obtained. These figures are for the purified products and do not take into consideration the residues left in the mother liquors.

0.0556 gm. copper salt was dissolved and made up to 25 cc.; 5 cc. samples gave 1.45 and 1.46 cc. of nitrogen at 22° and 743 mm.; average per cent amino nitrogen found 7.16; theory 7.19.

A portion of this copper salt was converted into the free acid.

0.0593 gm. was dissolved in water and made up to 25 cc.; 5-cc. samples gave 2.31 and 2.28 cc. of nitrogen at 25° and 744 mm.; average per cent amino nitrogen found, 10.51; theory 10.52.

The glutaminic acid hydrochloride melted at 197.5°.

0.0904 gm. was dissolved in water and made up to 10 cc.; 2-cc. samples gave 2.55 and 2.58 cc. of nitrogen at 25° and 736 mm.; average per cent amino nitrogen found 7.62; theory 7.63.

Estimation of pyrrolidon carboxylic acid

Foreman (6) has suggested a method for estimating the pyrrolidon carboxylic acid present by taking advantage of the fact that on boiling with hydrochloric acid it is converted into glutaminic acid with a corresponding increase in amino nitrogen.

A portion of the gum obtained in the above acetic acid extraction was analyzed for amino nitrogen.

0.0564 gm. gave 4.01 cc. of nitrogen at 23.5° and 742 mm. This corresponds to 3.88 per cent, or 1.971 gm. of amino nitrogen in 50.8 gm. of gum.

A portion of this gum was dissolved in water.

1 cc. of this solution gave 6.19 cc. of nitrogen at 24.5° and 753 mm. corresponding to 3.413 mgm. of amino nitrogen.

A 5-cc. portion was saturated in the cold in a small glass bulb with hydrochloric acid gas, a condenser was attached and the solution boiled for 15 hours. The contents of the bulb were diluted to 25 cc. and analyzed for amino nitrogen.

A 2.5-cc. sample gave 3.42 cc. of nitrogen at 24.5° and 744 mm. This corresponds to 3.724 mgm. per cubic centimeter, or 9.12 per cent of the total amino nitrogen.

The original 50.8 gm. of gum contained 1.971 gm. of amino nitrogen. Hence there would have been for the whole quantity of gum a total increase of 0.1798 gm. corresponding to a content of 1.657 gm. of pyrrolidon carboxylic acid.

This estimation was checked by extracting the pyrrolidon carboxylic acid from the gum with absolute alcohol. A portion of the gum weighing 12.65

gm. was triturated with absolute alcohol. The gum obtained by boiling off the alcohol was dissolved and made up to 50 cc. Of this 4 cc. was diluted to 25 cc.

1 cc. gave 0.72 cc. of nitrogen at 23.5° and 742 mm., corresponding to a total amino nitrogen content of 0.1228 gm. for the 12.65 gm. of the original gum.

Five cc. were hydrolyzed as before and made up to 25 cc.

1 cc. gave 1.30 cc. of nitrogen at 23.5° and 742 mm. This corresponds to a total of 0.1774 gm. of amino nitrogen in the 12.65 gm. of gum, or an increase of 0.0546 gm.

This is equivalent to a content of 2.0214 gm. of pyrrolidon carboxylic acid in the original gum as compared with 1.657 gm. by the former determination.

A 40-cc. portion of the solution of the gum was saturated with dry hydrochloric acid and boiled for 16 hours. After filtering off a flocculent precipitate, treating with animal charcoal and concentrating *in vacuo* it was seeded with glutaminic acid hydrochloride and placed in the refrigerator. After standing a few days a small crop of crystals was obtained, which in melting point and appearance under the microscope appeared to be identical with glutaminic acid hydrochloride. While it is possible that this arose from glutaminic acid dissolved by the acetic acid, it is more likely that it was formed from the pyrrolidon carboxylic acid since treatment of the gum directly without previous hydrolysis failed to yield anything resembling this compound.

DISCUSSION

This preliminary work shows several things of interest. In the first place, it shows that both glutaminic and aspartic acids are obtainable from peat by the methods employed. It also indicates the presence of pyrrolidon carboxylic acid, a fact which, of course, would be expected if glutaminic acid were present.

It is also apparent that while Foreman's method is applicable in a general way, certain details must be changed if it is to give satisfactory results with peat. This statement is true regarding the application to peat of any method devised for pure proteins. The presence of a comparatively high content of inorganic material and of humic organic substances introduces complications which make it necessary to make more or less extensive changes. In the present instance, for example, it becomes advisable to concentrate the original hydrolysate and saturate it with hydrochloric acid gas in order to remove as much of the inorganic matter as possible. But even then subsequent precipitates with the various reagents, such as calcium hydrate, phosphotungstic acid and silver sulfate are abnormally large as compared with those obtained in working up pure protein material. It is correspondingly difficult to wash them clean and at best they probably carry down more or less organic material which is ordinarily soluble.

The separation of the aspartic from the glutaminic acid in the acetic-acid-insoluble residue was not as sharp as was hoped for. The copper aspartate seemed to crystallize out almost completely in the first crop, for a further concentration yielded a very small amount of the light blue needles. When the glutaminic acid had been removed as completely as possible, however, the residue was again worked up for copper aspartate and a considerable quantity obtained. Carrying out the separation in the reverse order yielded no better results, as the glutaminic acid hydrochloride failed to crystallize readily and a humus-like precipitate came down in its place. It is possible that there is present some hydroxyglutaminic acid which complicates matters at this point.

The work is being continued along the lines suggested by this preliminary investigation.

SUMMARY

Attempts to separate glutaminic acid directly from the hydrolysate of peat have failed.

The application of Foreman's method has resulted in the separation of both glutaminic and aspartic acids and the estimation of pyrrolidone carboxylic acid from this material.

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THE FIXATION OF ATMOSPHERIC NITROGEN BY INOCULATED SOYBEANS¹

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Although it has been shown repeatedly by pot experiments that leguminous crops infected with the proper bacteria take nitrogen from the air there are much less data concerning the amount of nitrogen taken from the soil and from the air by plants grown under field conditions. With this in view, the Wisconsin Experiment Station has attempted to determine the amount of nitrogen taken from the soil and from the air by various leguminous plants. The usual method followed has been to compare the amount of nitrogen in infected plants with that in the same kind of plants grown on the same type of soil, but not infected. The difference between the amount of nitrogen in the inoculated and in the uninoculated crop represents the nitrogen secured by the cooperation of the bacteria with the higher plant. This difference in the amount of nitrogen fixed in the inoculated and the uninoculated crops is usually less than the actual difference which would result if it were possible to prevent altogether any infection from taking place in the uninoculated crop. The effect on the fertility of the soil planted to inoculated legumes is not brought out as well by analysis as by the growth of a non-leguminous crop on the same soil the following year.

In this article data are presented to show the amount of nitrogen taken from the air and from the soil when Ito San soybeans are grown under field conditions with and without the bacteria. The distribution of the nitrogen in the various parts of the plant, i.e., tops, roots, and nodules, also has been considered.

EXPERIMENTAL

The soil for this experiment was a light "blow" sand, low in plant food, especially nitrogen. For more than 20 years no leguminous crop had been grown here and no fertilizer had been added. In the spring of 1919 Ito San soybeans were planted in rows about 15 inches apart. One-half of the field was seeded with soybeans plus bacteria, the other half with soybeans minus bacteria. Except for inoculation, all the soybeans received the same treatment from the time they were planted until they were harvested. About 40

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days after the time of planting, the soybeans plus the bacteria showed a remarkable difference from the uninoculated in size and color. They were almost twice as tall as the plants without bacteria and a much darker green. Figure 1 of plate 1 shows clearly the difference in growth of the inoculated and uninoculated soybeans.

Representative portions from the inoculated and uninoculated plots were measured off and sampled. All of the plants, including roots and nodules, were carefully dug up from an area of 137.5 square feet. The nodules were carefully picked off the roots and brushed free of soil. The roots, tops, and, in the case of the inoculated plants, the nodules, were allowed to dry and were then weighed. Table 1 gives the results of these analyses.

TABLE 1
Yield of soybeans when grown with and without bacteria on Plainfield sand

TREATMENT	YIELD PER ACRE, AIR-DRY		
	Tops	Roots	Nodules
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
1. With bacteria.....	2598	197	115
2. Without bacteria.....	811	145	
Gain due to inoculation.....	1787	52	115

The figures show in a striking manner the effect of this partnership of soybeans and bacteria on the yield of forage. Soybeans with nodules produced more than three times as much dry forage as did those without nodules. The benefit of inoculation, however, is not confined to an increase in the yield of tops; there is also a gain in the weight of the roots. The plants were analyzed for total nitrogen and the data are given in table 2. Inoculation caused a decided gain in total nitrogen and also a gain in the percentage of nitrogen.

TABLE 2
Amount and percentage of nitrogen in soybeans when grown with and without bacteria on Plainfield sand

TREATMENT	AMOUNT OF NITROGEN PER ACRE				PER CENT OF NITROGEN		
	Tops	Roots	Nodules	Total	Tops	Roots	Nodules
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
With bacteria.....	57.10	2.40	5.86	65.36	2.41	1.31	5.67
Without bacteria.....	7.46	0.87		8.33	1.02	0.65	
Gain due to bacteria.....	49.64	1.53	5.86	57.03	1.39	0.66	5.67

The percentage of nitrogen in the dried infected plant tops was 2.41, in the roots 1.31, and in the nodules 5.67, while the nitrogen in the dried uninoculated plant tops was 1.03 and in the roots 0.65. The point of chief interest in the figures of this table is the enormous increase in the amount of nitrogen

in the case of the inoculated plants. Soybeans from this plot with bacteria contained 65.36 pounds of nitrogen while soybeans from the plot without bacteria contained only 8.33 pounds of nitrogen. The amount of nitrogen in the treated plants was almost 8 times as great as that in the untreated plants. The growth of soybeans in partnership with the nodule bacteria resulted in a gain in the percentage of nitrogen in the plant and a gain in the total weight of the forage. Visible evidence of the value of inoculation on the yield of soybeans is given in plate 1 (fig. 2) and plate 2.

The amount of nitrogen taken from the air depends on many factors: completeness of inoculation of the crop, the type of soil, the rainfall, the temperature, etc. If the amount of nitrogen removed from the inoculated and uninoculated soils by the crop is equal, then the difference between these two figures gives the amount taken from the air. On the Plainfield sand this amount was 57.03 pounds for each acre, or in terms of protein, 356 pounds. Whether grown for feed or for fertilizer this high percentage of protein in the soybean is an actual gain to the farm. The inoculated soybeans have drawn more than 87 per cent of their nitrogen from the air.

THE RESIDUAL EFFECT OF INOCULATED AND UNINOCULATED SOYBEANS

The tops of the soybean plants were harvested. The roots of both crops were left in the soil; in the case of the inoculated roots this meant a great many nodules. The following spring these plots were seeded to rye. Here the effect of inoculation on the fertility of this soil is well illustrated. The rye on the soil which had grown inoculated soybeans the previous year produced a much more vigorous growth accompanied by a darker green color. This difference in growth and color between the inoculated and the uninoculated plots was clearly seen from a distance. Figure 1 of plate 3 is a photograph of this field showing a part of the inoculated and the uninoculated field. The variation in growth is apparent in the photograph, although this picture does not fully bring out the striking differences. A better idea of the difference in the growth of the rye is seen in figure 2 of the same plate.

SUMMARY

From the results of this field experiment with Ito San soybeans inoculated and uninoculated on Plainfield sand it may be concluded that:

1. Inoculation increased the yield of soybeans 1787 pounds per acre, or more than threefold.
2. Inoculation resulted in a net gain in nitrogen of approximately 57 pounds per acre. At the time of harvest, the inoculated soybeans contained 57.10 pounds of nitrogen in the tops, 2.40 pounds in the roots and 5.86 pounds in the nodules, while the tops of the uninoculated plants contained only 7.46 pounds, and the roots 0.87 pound. By far the greater part of the nitrogen

of inoculated soybeans, approximately 87 per cent of the total increase in nitrogen, is in the tops of the plants.

3. Aside of the gain in yield inoculation causes an increase in the percentage of nitrogen in the tops and roots.

4. Although the entire crop of soybeans, parts above ground, were cut and removed, the after effect of inoculation was clearly shown in the growth of rye the following year. The residue of soybean roots and nodules greatly benefited its growth.

PLATE 1

FIG. 1. Part of the inoculated and uninoculated plots of soybeans on plainfield sand.

FIG. 2. Soybeans from 137.5 square feet of the uninoculated and inoculated plots.



FIG. 1



FIG. 2

PLATE 2

TEN SOYBEAN PLANTS UNINOCULATED COMPARED WITH 10 PLANTS INOCULATED



PLATE 3

FIG. 1. Rye on plots which had grown soybeans the previous year; at the left the uninoculated plot, at the right the inoculated plot. The relative height of the rye may be judged from the persons in the field.

FIG. 2. Rye on plots which had grown soybeans the previous year; at the left 100 stalks from the inoculated plot, and at the right 100 stalks from the uninoculated plot.



FIG. 1



FIG. 2

FIELD TESTS ON THE INOCULATION OF CANNING PEAS¹

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INTRODUCTION

The results of field tests in 1918 with Alaska peas, inoculated and uninoculated, near Holmen, Wisconsin, showed clearly the beneficial effect of the legume bacteria. Although this poor sandy soil is not especially adapted to peas, it was found that well inoculated plants made a fair to good growth, while those without nodule bacteria were small with only 1 to 2 peas in a pod. This beneficial effect of inoculation under field conditions resulted in a more general use of cultures, especially in the neighborhood of Holmen. As might be expected, many of the farmers failed to find any noticeable gain in the growth of the inoculated plants. Apparently, in the fields where cultures failed to show any increased growth, the soil was either rich in available nitrogen or already well supplied with the legume bacteria.

It was found that in general inoculation of peas on poor sandy soil increased the yield; in rare cases a somewhat similar effect was noted on the rich loam soils. In the heavier clay types of soil no benefit was noted.

The Wisconsin Agricultural Experiment Station has studied some of the factors concerned with inoculation of peas, and some of the results are presented in this paper. The agents which influence the composition and yield of peas are of great importance. This is true both from the standpoint of peas for food and from the standpoint of peas in respect to soil fertility.

It was arranged in the spring of 1920 to investigate the effect of inoculation on peas grown in different sections of the state. The desire for more accurate information in regard to the nitrogen supply of peas prompted the analytical work. By means of total nitrogen analysis of the soil before and after growing a crop, as well as analysis of the pea plants, it is possible to measure the effect of peas on the nitrogen supply of soil. In order to get more exact data in regard to inoculation, special field plots of canning peas were grown in Chippewa and Dane counties.

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CHIPPEWA COUNTY

In coöperation with the "Big Four" Canning Company an experimental plot of 1 acre, near Stanley, was planted to inoculated and uninoculated peas. An area of heavy rich clay loam soil, slightly acid, sloping to the eastward and 5 by 32 rods in extent was selected. This plot was plowed and harrowed and then staked off into two equal strips with an unplanted strip 2 feet wide between. One of the half-acre plots was drilled with uninoculated Alaska peas and the other with the same variety inoculated. The plots were planted May 14.

At the time the peas were planted it was understood that this plot of land had never before been planted to peas, at least not in several years. However, several weeks after planting it was learned that the plot selected for peas had been planted to this crop for many years. There was a good development of plants over the entire plot, except for a small area where heavy rain drowned out about an equal number of peas in each section. The plot was examined on July 2 and an abundance of nodules found on the plants in both the uninoculated and inoculated parts.

No difference in size or color could be seen in the peas from the inoculated and uninoculated sections. The peas were harvested under careful supervision on July 12 and the plants from each part of the plot vined separately. The yield of shelled peas was as follows:

	<i>pounds</i>
Inoculated.....	750
Uninoculated.....	780

The weights of the peas agree with the observations on distribution of nodules; there was no benefit from inoculation in this soil. The slight differences shown could easily be due to differences in soil, stand, or handling. On account of the total lack of field differences no analyses of plant tissue were made.

DANE COUNTY

The soil selected for this plot is a rich Carrington silt loam soil near Windsor. The Truog acidity test showed that the soil of this plot has a lime requirement of about 3 tons per acre. No lime was used in the experiment.

After it was plowed and harrowed the plot was divided into two equal parts $\frac{1}{2}$ acre in size. Each of the small plots was 7.5 feet wide and 115 feet long. The seed used was the commercial Alaska pea. Each of the small plots was sown in nine drills 115 feet long. Seed was sown at the rate of 4 bushels to the acre. On April 17 the plot was planted; uninoculated peas were sown first, then inoculated peas. The plants were examined on May 26 (full blossom), June 12, June 16 and June 21. On May 26 a difference in the size of the plants in the two plots was evident. By June 12 the inoculated peas were a darker green and the difference in size was more marked. The

peas were harvested on June 22. At this time the majority of the pods had reached the proper stage for harvest. The difference in the size and color of the plants in the plots is well shown in figure 1 of plate 1. The relative size of the yield of vines with the pods is shown in figure 2 of the same plate. Each pile is from one-fourth of the plot, or 0.01 acre. The yield of green vines and pods from the two plots was determined by weight and is given in table 1.

TABLE 1
Total green weight of vines and pods from inoculated and uninoculated peas

	AREA	
	0.01 acre	1 acre
	<i>lbs.</i>	<i>lbs.</i>
1. Inoculated	189	18,900
2. Uninoculated	157	15,700
Gain due to inoculation	32	3,200
Gain, percentage	20.37	

Comparison of weights shows that there were 3200 pounds per acre more green weight of vines and pods on the inoculated than on the uninoculated plot. This difference amounts to 20.37 per cent.

The pods were picked from the two piles of vines shown in figure 2, weighed, and the peas shelled and weighed. Table 2 gives the weights of the pods and peas from inoculated and uninoculated plants.

TABLE 2
Total green weight per acre of inoculated and uninoculated peas and pods

	PODS	PEAS
	<i>lbs.</i>	<i>lbs.</i>
1. Inoculated	3,880	2,570
2. Uninoculated	3,805	2,370
Gain due to inoculation	75	200
Gain, percentage	1.97	8.44

There was a substantial increase in yield in the inoculated plot in vines, pods, and peas.

The shelled peas were graded and the yields of the various grades are shown in table 3. From the figures of this table it will be seen that the peas from inoculated plants are larger than those from the uninoculated. The increase in size results in a decided gain in weight of peas belonging to grades 3, 4 and 5. The benefit of inoculation of this comparatively rich soil is of two kinds; first more of the large size of peas, due no doubt to greater maturity, and second, an increase of 8.44 per cent in total yield (table 2).

TABLE 3
Proportion of various grades of peas from inoculated and uninoculated plots

	YIELD PER ACRE		
	Grades 1 and 2	Grade 3	Grades 4 and 5
	lbs.	lbs.	lbs.
1. Inoculated.....	321	1,350	829
2. Uninoculated.....	466	1,111	691
Decrease or increase.....	-145	+239	+138

EFFECT OF INOCULATION ON NITROGEN SUPPLY OF THE SOIL

Chemical analyses of the soil from both parts of the plot were made for acidity (Truog test), for nitrates, and for total nitrogen. The determinations of nitrates and total nitrogen were made on an air-dry basis. In each case determinations were made in triplicate and total nitrogen determinations were made on two sets of samples. One set of soil samples was taken when the peas were harvested on June 24, and another on August 26.

Nitrates

In both parts of the plot, the inoculated and the uninoculated, the nitrates were present in such small amounts that it was not possible to make a colorimetric determination. Only a trace was present.

Total nitrogen

In order to compare the effect of inoculated and uninoculated peas on the total amount of nitrogen in the soil, careful analyses were made of the soil at the beginning and after the peas were harvested. At the time of planting the soil contained 260 mgm. of nitrogen in 100 gm. of dry soil. Because of the difficulty in drawing representative samples, portions of soil were taken from at least 10 different places in each of the plots and from these a composite sample prepared. The results of the nitrogen analyses are given on the dry basis. In each case the figures of table 4 represent the average of at least three to four closely agreeing analyses. Almost 2 months after the crop was harvested, soil samples from these two plots were taken and the analyses repeated. Here again, the soil of the inoculated pea plot gave a decidedly higher nitrogen content than that from the uninoculated plot. From the data it is seen that there is a marked residual effect of the inoculation. The results are calculated on the basis of a layer of soil 4 inches deep over one acre, or 1,000,000 pounds (Hopkins). It was deemed only just to calculate nitrogen on an acre 4 inches rather than on the acre 8 inches because; (a) most of the nodule formation, root growth, and hence nitrogen fixation takes place in this upper 4 inches and not in the 4 inches below, and (b) for samples

of soil as taken were from this upper 4 inches. It is evident from the results of these analyses for total nitrogen that with this type of soil and with proper inoculation there is no less nitrogen in the soil after a good crop of peas has been grown, cut, and removed, than there was before the planting. It must be noted that the plants from both inoculated and uninoculated plots bore nodules; although there were more and larger nodules on the inoculated plants. In both cases, the nitrogen content of the soil after removal of the crop was at least equal to the amount of nitrogen in the original soil. At the same time

TABLE 4
Effect of peas on the nitrogen supply of the soil

ANALYZED JUNE 24	TOTAL NITROGEN IN 100 GM. OF SOIL	TOTAL NITROGEN IN 1 ACRE OF SOIL
	mgm.	lbs.
1. Inoculated	276.1	2761.0
2. Uninoculated	262.9	2629.0
Gain due to inoculation	13.2	132.0

a gain in the nitrogen in the soil from the inoculated plot showed the benefit of good inoculation, produced by use of pure cultures, as compared with natural inoculation from the soil. A good crop of peas with a total green weight of 18,900 pounds per acre and a total weight of 2,570 pounds of shelled peas was grown and removed with an increase in the residue of nitrogen in the soil.

EFFECT OF INOCULATION ON THE NITROGEN CONTENT OF PEAS

Total nitrogen determinations were made of the two samples of pods from the Windsor plots. The results of the nitrogen analyses are given below:

	Nitrogen in 100 gm. of dry tissue mgm.
Inoculated pods	2355
Uninoculated pods	2333
Gain due to inoculation	22

It is evident from the figures not only that inoculation increased the yield of pods but that there was a corresponding increase in the nitrogen content. This increase in nitrogen content is important to the farmers who feed the pea-vine silage from the viner station, for it means: (a) that more of the valuable fertilizer, nitrogen, is returned to their farms, and, in part, to the soil and (b) that the silage is more valuable as a feed because of its increased nitrogen content. The gain in nitrogen through inoculation, then, cannot be measured simply by analyses of the soil after the peas are cut, but also must include the increase of nitrogen in pea silage. Not only are there more

pounds of silage per acre, but each pound contains more nitrogen than silage from uninoculated peas.

HORTICULTURAL GARDEN EXPERIMENTS

In order to test the effect of the inoculation of peas on very fertile soil, two rows of peas were planted in the truck garden of the horticultural department at Madison. These rows, 178 feet long and 18 inches apart, were planted on May 29 to Alaska peas. The soil is a heavy silt loam that has been heavily manured with stable manure for many years. The Truog test for soil acidity shows a neutral reaction. According to analyses this rich black garden soil contains about 0.410 per cent of nitrogen. The peas in the east row were uninoculated and the peas in the west row were inoculated.

The plants developed normally but the stand was much reduced by pigeons. In many places the peas were scratched out of the soil. About two-thirds of a stand remained in each row. On June 28, four weeks after planting, the plants started to form pods. At this time there was no apparent difference in size or color of the vines in the two rows. On July 13, almost 7 weeks after

TABLE 5

Green weight of 100 inoculated and 100 uninoculated pea plants from the horticultural plot

	VINES	PODS	PEAS
	gm.	gm.	gm.
Inoculated plants.....	552	394	158
Uninoculated plants*.....	360	133	145
Gain due to inoculation.....	192	261	13
Gain in percentage.....	53.33	196	8.96

* Many immature pods.

planting, about 25 representative plants were selected from each row. The uninoculated peas showed a few small nodules and on certain plants the roots were soft and black—perhaps because of “damping off” fungi. The inoculated plants showed a better root system and more and larger nodules than the uninoculated plants. Little if any disease could be found on the roots of the inoculated plants. From each of these two groups of plants ten were selected as an average to be weighed and photographed. The tops and roots are shown in plates 2 and 3. The weight of ten average uninoculated plants was 123 gm. and of ten inoculated plants 143 gm.

On July 15 the entire two rows were harvested and 100 plants were selected from each by a garden laborer who was not familiar with the plan of the experiment. He was asked to select 100 good plants from each row. These groups of plants were weighed and analyzed (table 5).

The effect of inoculation here is similar to that of the previous experiment. The greatest difference was noted in the vines and pods but there also was a marked difference in the yield of shelled peas.

Unfortunately the seeds of these peas were lost during drying. The analysis of the pods is shown below:

	Nitrogen in 100 gm. of dry tissue mgm.
Inoculated peas.	1853
Uninoculated peas.	1743
	<hr/>
Gain due to inoculation.	110

Because of the small area of peas used in this test no attempt was made to calculate the results in pounds per acre. It seems fair, however, to say that the results in general agree with those obtained at Windsor.

CONCLUSIONS

In the experiment at Stanley, with a heavy, rich, clay loam soil, slightly acid, which had been cropped to peas for years, inoculation apparently had no effect.

In the Windsor experiment with a rich silt loam soil, unlimed and acid inoculation has been shown to be beneficial. Inoculation has caused an increase in the total yield of pea plants, in the yield of peas, and in the percentage of nitrogen. An increase in the weight of shelled peas means an increase in cash returns. An increase in the nitrogen content of the plant means an increase in the value of the plants or pea silage as feed. Analyses of the soil in the Windsor plot have shown that together with an increased crop of peas there is a gain in nitrogen in the residual soil where inoculated plants have been grown, or that the inoculated peas have acted as an efficient nitrogenous fertilizer. Nitrogen has been added not only for the benefit of the pea crop itself but also for the subsequent crops on that soil.

The experiment on the Horticultural Garden plot has indicated that these favorable results of inoculation are not confined to the acid silt loam soil. The soil in this case was neutral in reaction, and had been heavily manured for years. Again inoculation produced an increase in yield and in the percentage of nitrogen in the plants.

PLATE 1

FIG. 1. Alaska Peas on Carrington silt loam soil. The signs are equally distant from the soil.

FIG. 2. Alaska peas on Carrington silt loam soil. The piles of peas have approximately the same diameter.



FIG. 1



FIG. 2

PLATE 2

PEAS FROM THE HORTICULTURAL PLOT AT MADISON, SHOWING ROOTS AND TOPS; INOCULATED
AND UNINOCULATED

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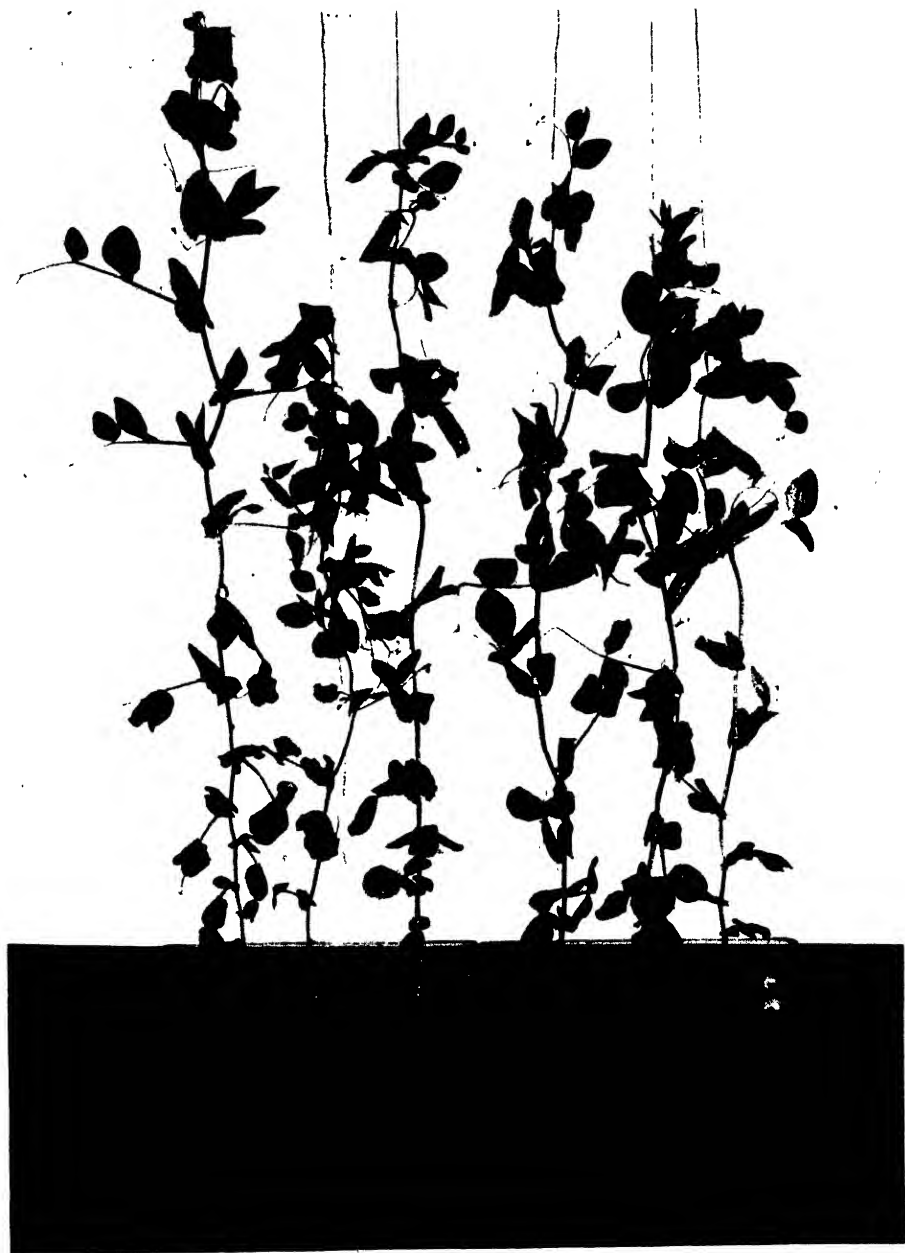


PLATE 3

PEAS FROM THE HORTICULTURAL PLOT AT MADISON, SHOWING ROOTS, INOCULATED AND
UNINOCULATED

E. B. F&ED, W. H. WRIGHT AND W. C. FRAZIER



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